CHARLES UNIVERSITY IN PRAGUE

FACULTY OF PHYSICAL EDUCATION AND SPORT

Hyperbaric Oxygen Therapy In Relation To Production Of Free Radicals " Oxidative Stress"

DIPLOMA THESIS

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Abstract

Objectives. The purpose of this Thesis is to Create a Critical Review that describes the methods, results, and conclusions of a literature review of the benefits and harms of hyperbaric oxygen therapy (HBOT) and the relation to the free radical production "Oxidative Stress", the usage of the (HBOT) for brain injury, cerebral stroke, and some neurological disorders.

Selection Criteria. We independently assessed each title and abstract using Predetermined inclusion criteria based on intervention, population, outcome measures, and study design criteria. Full papers, reports, and meeting abstracts that met inclusion criteria were retrieved and reviewed independently.

Main Results. Determine whether the benefits of HBOT outweigh the potential harms. For other types of brain injury, no good- or fair-quality studies were found. No controlled trial of HBOT was designed to measure mortality in stroke patients, and the best studies found some what improvement in neurological outcomes. Evidence about the type, frequency, and severity of adverse events in actual practice is inadequate. Reporting of adverse effects was limited, and no study was designed specifically to assess adverse effects. Evidence from well-conducted clinical studies is limited. The balance of benefits and harms of HBOT for brain injury, cerebral palsy, or stroke has not been adequately studied.

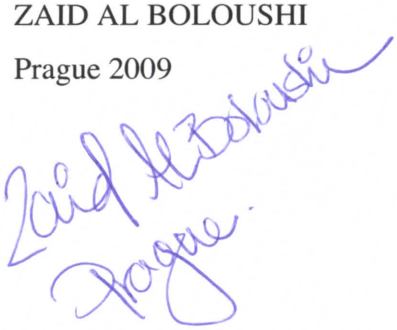
Key words: HBOT, HBO, Hyperbaric oxygenation, Free radicals, oxidative stress, Stroke, cerebral stroke, brain injury, stroke.

Declaration

Herewith I declare that I worked on this thesis on my own and independently I researched the list of literature and data's, which included in this work. I elaborated using the literatures listed and attached in the reference section and the knowledge I have gain during my study time at Charles University.

ZAID AL BOLOUSHI

Prague 2009



Dedication

I dedicate my diploma to the divinity, then to my beloved family. Further more to my university, Professors and Tauter, Colleagues, and finally to Scientific Community for the future researchers.

Acknowledgment

First, deep gratitude goes firstly to my supervisor PhDr. Michaela Prokešova, for her continuous support who patiently guided encouraged and supported me throughout my whole time to write the diploma thesis, she showed Care and she was always there to listen and to give me an advice.

I would like also to thank the crew in Kladno Hospital the Hyperbaric Chamber Department, Specially to MUDr. Jana Sůvová and Mr. Jan Stanék

I forward grateful thank to the department of physiotherapy, all members and teachers for their great support and guide through my master program I forward grateful thanks to Doc. MUDr. František Véle, CSc. who explained us great knowledge during my study time in Faculty of Physical Education and Sport, Charles University, In Prague. Which have formed our thinking about the subjects and especially from the Psychological and Philosophical aspects and added new sight for us.

I am grateful to all my teachers who to taught us through my program at Charles University,

I thanks also my colleagues and friend how helped me discuss ideas about my work. Last but not least I would like to thank my family for their support specially my mother who impassion me and is still supporting me.

Herewith I primate to lend my diploma thesis, for study purpose, I encourage my Colleagues' to quote and cite from this thesis.

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List of Equations

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List of abbreviations

HBO: Hyperbaric Oxygen
HBOT: Hyperbaric Oxygen therapy
WHO: World Health Organization
CNS: Central Nervous System
TBI: Traumatic brain injury
TOSAT: Acute Stroke Treatment
RIND: Reversible Ischemic Neurological Deficit
PRIND: Prolonged Reversible Ischemic Neurological Deficit
ICH: Internal Cerebral Hemorrhage
SAH: Subarachnoid Hemorrhage
ISCH: Spontaneous Intracerebral Hemorrhage
DM: Diabetes Mellitus
CT: Computer Tomography
MRI: Magnetic Resonance Imaging
BP: Blood Pressure
CI: Confidence Interval
O2: Oxygen
CO2: Carbon monoxide
BBB: Blood Brain Barrier
TLE: Temporal Lobe Epilepsy
CBF: Cerebrospinal Fluid
AF: Arterial Fibrillation
PFO: Patent Foramen Ovale
ABCD score: (age, blood pressure, clinical features, duration)
ABCD 2 score: (age, blood pressure, clinical features, duration) 2= Diabetes Mellitus

EDFR: Endothelium Derived Relaxing Factor
NO: Nitric Oxide
LDL: Low-Density Lipoprotein
ECM: Extracellular Matrix
SMCs: Smooth Muscle Cells
ACC: American College of Cardiology
AHA: American Heart Association
CVDs: Cardiovascular Disease's
CVA: Cardiovascular Accident
mRNA: Messenger Ribonucleic Acid
(TPA, PLAT, rtPA): Tissue Plasmogenic Activator
AIS: Acute Ischemic Stroke
SOR: Strength of Recommendation
ST: International Stroke Trails
CAST: Chinese Acute Stroke Trail
MCAO: Middle Cerebral Artery Occlusion
EC: Endothelial Cells
IL-1: Interleukin-1
TNF-alpha: Tumor Necrosis Factor
MCP-1: Monocyte Chemoattractant Protein-1
NPY: Neuropeptide Y
NMDA: N- methyl- D -aspartate
AMPA: alpha-amino-3-hydroxy-5-methyl-4- propionate
SNAP25: Synaptosomal-associated protein 25
ROS: Reactive Oxygen Species
RNS: Reactive Nitrogen Species
FFA: Free Fatty Acids
PAF: Platelet-Activating Factor
ATP: Adenosine Triphosphate
ADP: Adenosine Diphosphate
BDNF: Brain-Derived Neurotrophic Factor
CNTF: Ciliary Neurotrophic Factor
EPR: Electron Paramagnetic Resonance
HSPs: Heat Shock Protein Family
OH: Hydroxyl
NOS: Nitric Oxide Syntheses
nNOS: Neuronal Nitric Oxide Syntheses

iNOS: Inducible NOS
eNOS: Endothelial NOS
1O2: Singlet Oxygen
H2O2: Hydrogen Peroxide
ANT: Adenine Nucleotide Transporter
Pi: Inorganic Phosphate
PC: Phosphate Carrier
ONOO: Peroxynitrite
PRX III: Peroxiredoxin III
GSH: Glutathione
GSSG: Glutathione Disulfide
GR: Glutathione Reductase
TH: Transhydrogenase
NADH: N-methyl-D- aspartate
UPR: Unfolded Protein Response
COPD: Chronic Obstructive Pulmonary Disorder
RA: Rheumatoid Arthritis
OA: Osteoarthritis
NANC: Nonadrenergic, Noncholinergic
ATA: Atmosphere Absolute
UHMS: Undersea and Hyperbaric Medicine and Society
P: Partial pressure
pO2: Partial pressure of Oxygen
pAO2: Partial pressure of Oxygen in alveoli
paO2: Partial pressure of Oxygen in arterial blood
pvO2: Partial pressure of Oxygen in venous blood
mmHg: millimeter of mercury
in Hg: inches of mercury
psi: pounds per square inch
bar: bar
kg/cm2: kilogram per square meter
fsw: feet of seawater
msw: meters of seawater
atm: atmosphere
DCS: Decompression Sickness
RBC: Red Blood Cell
RBCs: Red Blood Cells
MCA: Middle Cerebral Artery
EEG: Electroencephalograph
RCTs: Randomized Controlled Trials
NRCTs: Nonrandomized Controlled Trials
AChE : Acetylcholineserase
PFO: patent foramen ovale

1 Introduction

Adopting it as a therapeutic agent from a deep sea commercial diving and from navies around the glob. The medical profession has been administrating the Hyperbaric Oxygenation as a part of treatment for certain criteria and patients, whom they could go under this therapy. We believe as any other type of therapy which was brought to us through our ancestors and Science, that every therapeutic tool by the end of the day we want to improve our patients outcome, patient who suffered from traumatic brain injuries, stroke, neurological disorders, so many doctors have given up on them, and they decided that their patient have to accept their faith. We believe that we could help patient more than only administrating medical and pharmaceutical medications, which goes hand in hand with other type of therapeutic methods, the medical field in this new era consist of multidisciplinary work. The concept of the HBOT comes from the oxygen and diving. As I was undergoing my diving and taking my course I came into mentioning the HBOT as a main treatment for nitrogen necrosis in divers. We thought about the possibility of the oxygen intake in other disorders and specifically neurological patients. So we decided after the encouragement of my supervisor PhDr. Michaela Prokešová and Colleagues' from the Kladno City *(See Acknowledgment)* to take this topic as my thesis, and since the first part was already done by my Colleague Tufikameni Jason. We decided to answer the questions, which was related to the HBOT and the relation to the free radicals productions?

This evidence report describes the methods, results, and conclusions of a literature review on the use of hyperbaric oxygen therapy (HBOT) to treat manifestations of brain injury, and stroke in humans. And the relation of production of free radicals during or after the exposure to the HBOT. Hyperbaric oxygen therapy is the administration of high concentrations of oxygen within a pressurized chamber. HBOT has become the definitive therapy for patients with decompression illness, gas embolism, and severe, acute carbon monoxide poisoning and is a widely accepted treatment for osteoradionecrosis, soft tissue radionecrosis, wound healing, and several

other conditions. However, the role of HBOT in the treatment of patients with brain injuries, stroke and some neurological disorders is controversial. *Hyperbaric Oxygen Therapy* which is highly effective 300-year-old treatment modality called hyperbaric oxygenation, its is a very important medical tool of great potential and importance. HBOT Which is an abbreviation for the Hyperbaric Oxygen therapy, is a method of restoring wellness and utilization of the principles pertaining to gases and their physiological effects. These effects can reverse pathological states in which the fact that too little oxygen is reaching the cells (a condition which called hypoxia). When HBOT is used, the oxygen molecules are up to ten times more abundant than they are in air. Hardly any more oxygen to be imparted to the hemoglobin. But the bulk of the blood, called blood plasma, which essentially water, dissolves some oxygen.

In this thesis we would like to suppress on the HBOT we will go through the studies and the experimental procedures which were done with the use of HBOT, we will review the production of free radicals " Oxidative Stress" which the new era of Scientific Community, are separated into two main criteria. Either they are with the treatment or contradicting it. We will go in a systemic way through a basic history and principle of the HBOT, then we will review some basic concept of the Free Radicals " Oxidative Stress", we will follow up with an Antioxidants, then we will go through a Basic aspect of Stork, types of stroke and it Mechanisms.

We will try to answer some of the Scientific Questions, which are debating the use of HBOT in modern medicine and specifically in Stroke, and some other neurological disorders. The questions are based on:

What is the relation between HBOT and free radicals "Oxidative stress"?

Dose the Dosage and exposure time in the chamber enhance the production of free radicals?

Dose antioxidant and other medicaments maintain the production of free radicals?

2 Aims and Goals

2.1 Aim:

To find the evidence in relation between the hyperbaric oxygen therapy and the production of free radical, via critical review.

2.2 Goals:

G1: *Propose and critically review the hyperbaric medicine, definition, and its mechanism of action and general indications.*

G2: *Critical review literatures, attempting to treat different neurological disorders via HBOT, as well some other studies in relation to the production of free radicals "oxidative stress".*

G3: *Critical review of literatures the Correlation of hyperbaric medicine in different treatment, a review of the main studies which were done for neurological disorders.*

G4: *Critical review of literatures, on the oxidative stress and relations to aging and stroke.*

G5: *I will review various literatures about Strokes, types of stroke and risk factors and pathology and mechanism of stroke.*

Further more towards the goals of this thesis is to search and find if there is proper dosage and duration HBOT in stroke patient or overall different neurological disorders, if any organization or community sit a specific protocol which is applied been followed by medical practitioners'.

3 General Part

In this thesis we decided to carry out a critical review, will be reviewing the articles systematically and we will analysis the outcome's and the result's of the studies. We will be reviewing articles of patients and studies, which were experimental on animals and patients', the studies, which were controlled, uncontrolled, and randomized. We will review the HBOT and its effects according to the UHMS *Under Sea and Medical Society,* and the international standards. The analyses of individual studies reviewed for quality and consistent evidence for each key question is based on the sufficient Evidence, Validity of the information also regarded Author's opinion and advices due to there experience's in the case of the hyperbaric oxygenation we also included arguments of these anthers about hyperbaric oxygenation and oxidative stress. The quality of all trials in the review was assessed using a list of items indicating components of internal validity. We modified the standard checklists to address issues of particular importance in studies of HBOT. For randomized controlled trials (RCTs) and nonrandomized controlled trials (NRCTs).

3.1 History

Hyperbaric therapy was first documented in 1662, when Henshaw built the first hyperbaric chamber, or "domicilium", since this time, report of beneficial effects from increased pressure have increased and in 1877, chambers were used widely for many conditions, though there was little scientific rationale or evidence, in 1879, the surgical application of hyperbaric therapy in prolonging safe anesthesia was realized and explored. In 1927, Cunningham reported improvement in circulatory disorders at sea level and deterioration at altitude, and a patient who was grateful to Cunningham for his recovery after HBOT, built the huge 'steel ball hospital' chamber but this was closed when Cunningham failed to produce evidence for it use (Jain, 2004)

Early chambers used compressed air rather than oxygen, due to early reports of oxygen toxicity. Drager was the first to explore the use of pressurized oxygen in decompression sickness, and Behnke and Shaw put his protocols into practice in the late 1930s. Researcher conducted by the US military after the Second World War brought greater knowledge about survivable pressures. As a result, the use of HBO increased, and thought the late 1950s and early 1960s, HBO was used to potentiate radiotherapy effects, prolong circulatory arrest during surgery, and to treat anaerobic infections and carbon monoxide poisoning, unfortunately HBO has also been used without a solid evidence base in condition such as dementia, emphysema and arthritis. Concerns about the lack of scientific progress and regulation led the UHMS "*Undersea and Hyperbaric Medicine and Society"* to form a Committee on Hyperbaric Oxygen Therapy in the late 1970w, which is now the international authority on HBO (Gill & Bell, 2004; Jain, 2004).

Oxygen free radical's are products of normal cellular oxidation- reduction processes. Under conditions of Hyperoxia their production increases markedly. The nature of the oxygen molecule makes it susceptible to univalent reductions in the cells to form a superoxide anion (O2-) a highly reactive, cytotoxic free radical, in turn, other reaction product of oxygen metabolism, including hydrogen peroxide (H2O2), hydroxyl (OH'), and singlet oxygen (1O2), can be formed. These short-lived forms are capable of oxidizing the sulfhydryl (SH) groups of enzymes, interact with DNA and promots lipoperoxidation of cellular membranes. Animals studies showing the effect of HBOT in raiding the cerebral peroxide content and correlating with CNS tocxicity (Jain, 2004)

There is a hypothesis of oxygen toxicity which was reproduced from [Chance and Boverix 1978, (Jain, 2004)].

Hyperbaric Oxygen Therapy which is highly effective 300-year-old treatment modality called hyperbaric oxygenation, its is a very important medical tool of great potential and importance. HBOT Which is an abbreviation for the Hyperbaric Oxygen therapy, is a method of restoring

wellness and utilization of the principles pertaining to gases and their physiological effects. These effects can reverse pathological states in which the fact that too little oxygen is reaching the cells (a condition which called hypoxia).

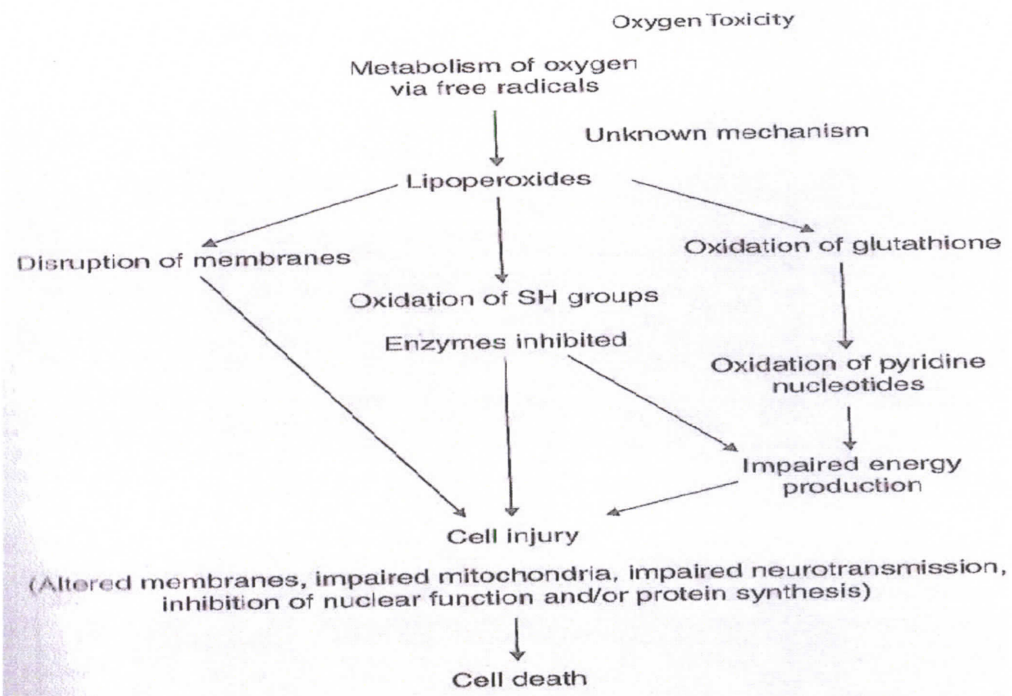

Figure 1: The hypothesis schema of the oxygen toxicity (Jain, 2004)

Table 1: Animal experimental studies that were done to see effect of HBO on Brain Lipoperoxide Levels: (Jain, 2004)

Auther	Pressure	Effects
Zirkle et al (1965)	4 ATA	Clinical levels of CNS toxicity correlated with elevated lipoperoxide content; convulsions were considered to be due to raised AChE (acetylcholineserase) activity
Galvin (1962)	2 ATA	Cerebral peroxide level not elevated; no difference in the peroxide level between convulsing and nonconvulsing animals
Jerrett et al (1973)	5 ATA	H2O2 level were elevated except in those animals given supplemental alpha-tocopherol
Yusa et al (1987)	3 ATA	Rise of H2O2 level in brain by 300% when symptoms of CNS toxicity became apparent
Torbati et al (1992)	3 ATA	Direct demonstration of reactive oxygen species before onset of CNS convulsion

3.2 Critical review

We will began by explaining some basic terms of HBOT and how it works. It is easier to understand the concept of HBOT if we understand some of the basic terms. One of these terms is atmospheric pressure. The air around us exerts pressure because its weight, that is being pulled towards the earth center by gravity. Atmospheric pressure decreases as one climbs above sea level because the column of air over one's head weighs less the higher one goes. As one dives below the surface of the ocean, pressure increases because of the column of water above one's head weighs more than air. This water pressure is called *hydrostatic pressure*.

As we will go through this paper work we will try to explain some basic terms and knowledge of the free radical's and how its sets on attacking our

bodies. The free radicals are normally produced in our body via the *Mitochondria , which is the power of the cell in production and functioning organ.* A free radical defined as; any species capable of independent existence that contains one or more unpaired electrons. (e.g. of free radicals are Superoxide (O2) and hydroxyl (OH) both oxygen-centered radicals, most biological molecules are non-radical containing only paired electrons, when non-radicals and radicals react together free radical chain reaction often result (Slater, 1984; Thomas, 1998; Wu & Cederbaum, 2003).

We will then go through the deep analyzing of the free radical's risk factors, which involve aging and DNA damage. We will further expose the antioxidants and their effects on *vivo*. And last but not least we will explain the stroke. Stroke was defined according to the World Health Organization (WHO), criteria as rapidly developed clinical signs of focal disturbance of cerebral function, lasting for 24 h, or leading to death, with no other apparent cause than cerebrovascular disease. (Abo K, 1980). We would like to answer the questions which were mentioned earlier and if the HBOT is harmful.

3.2.1 Hyperbaric medicine (Hyperbaric oxygenation therapy)

INTRODUCTION OF HBOT

As well known, the origins and development of hyperbaric medicine are closely tied to the history of diving medicine. While the attraction of the deep sea's are easily understood, it was various unpleasant physical consequences of venturing beneath the surface of the world's oceans that led directly to the many applications of compressed gas therapy in modern medicine.

Hyperbaric Oxygen Therapy is defined by the Committee on Hyperbaric Medicine as " A mode of medical treatment in which the patient is entirely enclosed in a pressure chamber and breathes 100% Oxygen at a pressure

greater than 1 atmosphere absolute (ATA) ". The unit of Pressure is ATA, 1 ATA is equivalent to 760 mm of Mercury or pressure at sea level.

The application of HBO depends on the physical properties of gases under pressure, specifically, oxygen at pressure greater than 1atm. Oxygen is essential in a variety of enzymatic, biochemical, and physiologic interactions that promote normal cellular respiration and tissue function. Mono-oxygenase, intradioxygenase, and interdioxygenase are specific enzymes that recruit oxygen as a cofactor to perform required biologic processes. Collagen deposition and synthesis depend on an oxygen dependent prolyl-hydroxylase hydroxylation of proline. Angiogenesis and epithlization are also oxygen dependent (Jain, 2004).

Hyperbaric oxygen (HBO) therapy can be defined as intermittent inhalation of 100% oxygen, at a pressure greater than 1 atmospheric absolute (ATA), it can increase oxygen tension in arterial blood and tissue, improves the cellular oxygen supply by raising the tissue-cellular diffusion gradient, promote angiogenesis, and decrease the diameter of bubbles in gas embolism (Gill & Bell, 2004; W. Liu, et al., 2008)

PHYSICAL, PHYSIOLOGICAL, AND BIOCHEMICAL ASPECTS OF HBO

Oxygen is the most prevalent and most important element on earth. A Brief description of how oxygen is transported and the basic physical law governing its behavior will be useful to understand the principle of the HBOT.

Partial pressure	**P**
Partial pressure of Oxygen	**pO2**
Partial pressure of Oxygen in alveoli	**pAO2**
Partial pressure of Oxygen in arterial blood	**paO2**
Partial pressure of Oxygen in venous blood	**pvO2**

mmHg millimeter of mercury

in Hg inches of mercury

psi pounds per square inch

bar bar

kg/cm2 kilogram per square meter

fsw, msw feet or meters of seawater

atm atmosphere

ATA atmospheric absolute

The atmospheric is a gas mixture containing by volume 20.94% oxygen, 78.08% nitrogen, and 0.04% CO2 and traces of other gases. For practical purposes we will consider the air to be mixture of 21% oxygen and 79% nitrogen. The total pressure of this mixture at sea level is 760 millimeters of mercury (mmHg).

Theoretical consideration of hyperbaric medicine

The only absolute atmospheric pressures are those measured by a mercury barometer. In contrast, gauge pressures are measure of difference between the pressure in a chamber and the surrounding atmospheric pressure. To convert pressures as measured by a gauge to ATA requires addition of the barometric pressures.

Dalton's law states that in a gas mixture, each gas exerts its pressure according to its proportion of the total volume.

Partial pressure of gas= (absolute pressure) x (proportion of total volume of gas).

Thus, the partial pressure of oxygen (pO2) in air is

(760) X (21/100)= 160 mmHg.

Boyel's law states that at a constant temperature, the pressure and volume of a gas are inversely proportional. This is the basis for many aspects of hyperbaric therapy, including a slight increase in chamber temperature during treatment; and the phenomenon known as "squeeze", occurring when blocked Eustachian tubes prevent equalization of gas pressure, resulting in painful compression of gas in the middle ear. In patients who cannot independently achieve pressure equalization, the placement of tympanostomy tubes should be considered to provide a channel between

inner and outer ear spaces. Similarly, trapped gas can enlarge dangerously during compression, such as in the rare examples of a pneumothorax occurring at pressure.

Henry's law states that the amount of gas dissolved in a liquid or tissue is proportional to the partial pressure of that gas in contact with the liquid or tissue. This is the basis for increased tissue oxygen tension with HBO treatment. However, it also has implications for decompression needs in the air-breathing attendants in multiplace chambers, as their tissue concentration of inert gases (particularly nitrogen) will also be increased. This nitrogen will dissolve in the blood and may come out of solution and form arterial gas emboli during depressurization.

Most oxygen carried in the blood is bound to hemoglobin, which is 97% saturated at atmospheric pressure. Some oxygen is however carried in solution, and this portion is increased at pressure due to Henry's law, maximizing tissue oxygenation. When breathing normobaric air, arterial oxygen tension is approximately 100 mmHg, and tissue oxygen tension approximately 55 mmHg. However 100 % oxygen at 3 ATA can increase arterial oxygen tension to 2000 mmHg, and tissue oxygen tensions to around 500 mmHg, allowing delivery of 60ml oxygen per liter of blood (compared to 3ml/l at atmospheric pressure), which is sufficient to support resting tissues without a contribution from hemoglobin, as the oxygen is in solution, it can reach physically obstructed areas where red blood cells cannot pass, and can also enable tissue oxygenation even with impaired hemoglobin oxygen carriage, such as in carbon monoxide poisoning and severe anemia. (Gill & Bell, 2004; Jain, 2004)

The major site of utilization of molecular oxygen within the average cell is the mitochondria, which account for about 80%, while 20% is used by other sub cellular organs, such as microsoms, nucleus, the plasma membrane, etc. Oxygen combines with electrons derived from various substrates to release free energy. This energy is used to pump H+ ions from the inside to the outside of the mitochondria against an electrochemical gradients. As H+ ions diffuse back, free energy is made available to

phosphorylates adenosine diphosphate (ADP), and adenosine triphosphate (ATP) is generated. Only a minute amount of oxygen required for the normal intracellular chemical reactions to take place. The respiratory enzyme system is so geared that when tissue pO2 is more than 1-3 mmHg, oxygen availability is no longer a limiting factor in the rate of chemical reaction.

Density

As barometric pressure rises there is an increase density of gas breathed, the effect of increased density on resting ventilation is negligible within the range of the 1.5-2.5 ATA usually used in HBO. However with physical exertion in patients with decreased respiratory reserves or respiratory obstruction, increased density may case gas flow problems.

Temperature

The temperature of the gas rise during compression and falls during decompression, according to Charle's law, if the volume remains constant, there is a direct relationship between absolute pressure and temperature.

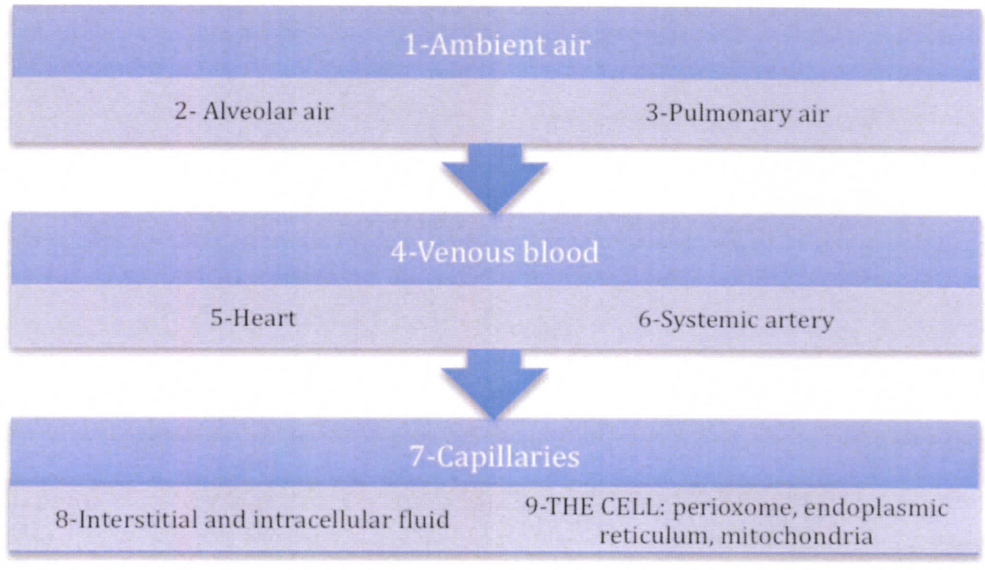

Figure 2: Physiology of oxygenation (Jain, 2004)

Ventilation phase

Oxygen is continuously absorbed into the blood, which moves through the lungs, at a ventilatory rate of 5 liters/min oxygen consumption of 250 ml/min. the alveolar oxygen tension is maintained at 104 mmHg. During moderate exercise the rate of alveolar ventilation increases fourfold to maintain this tension and about 1,000 ml of oxygen are absorbed per minutes. Carbon monoxide is being constantly formed in the body and discharged into the alveoli secretion is 40 mmHg. It is well know that partial pressure of alveolar CO2 (pCO2) increases directly in proportion to the rate of CO2 excretion and decreases in inverse proportion to alveolar ventilation (Jain, 2004)

HBO AND RETENTION OF CO2

When HBO results in venous blood being 100% saturated with oxygen, there is a rise in blood pCO2 and a shift of pH to the acid side. This is due to loss of hemoglobin available to transport CO2. This affects only the 20% of the venous content of CO2, which is transported by hemoglobin. Excess CO2 is transported by the H2CO3/HCO3 mechanism, as well by entering into physical solution in plasma. The elevation of cerebral venous pCO2 is of the order of 5-6 mmHg when venous hemoglobin is 100% saturated with oxygen. CO2 dose not continue to rise in venous blood and the tissues as long as the blood flow remains constant, and the presents no major problems. (Jain, 2004)

Tissue oxygen tension under HBO

Various factors relating to tissue oxygen tension under HBO:

- arterial pO2 is the maximum pO2 to which any tissue will be exposed, and plays a major part in determining the pO2 diffusion gradient driving oxygen into the tissues. Arterial pO2 depends on the inspired pO2.
- Arterial pO2 content is the total amount of oxygen available, it depends on the inspired oxygen and the blood hemoglobin level.
- Tissue blood flow regulates the delivery of oxygen to the tissue
- Tissue oxygen levels vary according to utilization of the available oxygen,

In typical tissue, arteriovenous oxygen difference rises to 350 mmHg when 100 % oxygen is breathed at 3 ATA if half reduces the blood flow to the tissues, the corresponding values of capillary pO2 will be 288 mmHg and 50 mmHg. Another factor is the vasoconstriction effect of HBO, which reduces the blood flow. Effective cellular oxygenation can be accomplished at very low rates of blood flow when arterial pO2 is very High(Jain, 2004)

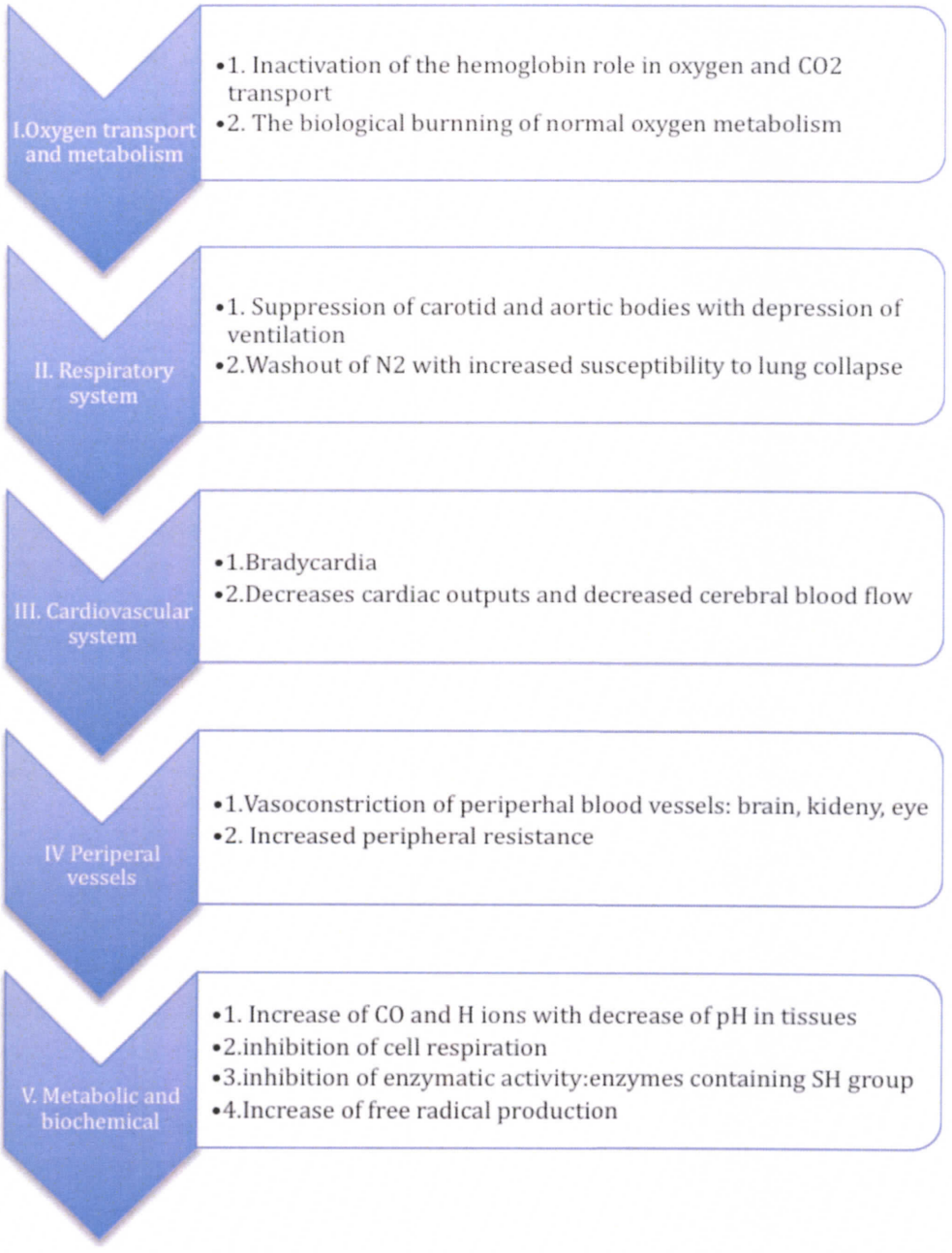

Figure 3: Effect of Hyperoxia (Jain, 2004)

EFFECT OF HBO TREATMENT ON PLASMA BLOOD VOLUME AND HEMOGLOBIN

Since oxygen is transported to the tissue in the blood stream, interputation of blood flow means that the amount of available oxygen to the cells falls to zero. Under these conditions, the rate of tissues utilization of oxygen blood is limited. The effect between hemoglobin reactions on transport of CO2 is known as Haldane effect, result from the fact that combination of oxygen with hemoglobin causes it to become stronger acid. This displaces CO2 from the blood in two ways:

- When there is more acid, hemoglobin has less of a tendency to combine with CO2 to form carbhemoglobin , much of the CO2 present in this form in the blood is thus displaced.
- The increase of acidiy of hemoglobin causes it to release an excess of H+ ions and these, in turn, bind with bicarbonate ions to form carbonic acid, which then dissociates into water and CO2, which is released from the blood into the alveoli.

Under a normal conditions with a good perfusion at rest, tissues require 5-6 mL per dL of oxygen this can be dissolved or hemoglobin-bonded oxygen. Increasing the pressure to 3 atm increases the blood oxygen (dissolved oxygen not carried by hemoglobin) to 6 mL per dL (Jain, 2004; Sheridan & Shank, 1999).

A classical study on the subject of life without blood was made by (Boerema I, 1959), the author lowered the level of hemoglobin in young pigs to 0,4% by exchanging the blood for plasma by venesection. The animals breathing oxygen at a pressure of 3 ATA in the hyperbaric chamber lived for 45 min with a level of hemoglobin that would be incompatible with life at atmospheric pressure. These animals were kept alive virtually without any hemoglobin. ECG showed no changes, and the circulation and the blood pressure remained spontaneously normal. Recovery was uneventful after reinfusion of blood prior to decompression to the surface. (Bockeria LA, 1979) reported use of hemodilutation up to 55%. HBO was able to provide adequate oxygenation during open-heart

surgery. (Koziner VB, 1981) replaced 94%-98% of the blood volume with dextran in cats and kept them alive for 8-9 h, breathing 100% oxygen.

Blood-Brain and Cerebrospinal Fluid–Brain Barriers

Blood-Brain Barrier *(BBB)*, and effect of HBO on the *(BBB)*

The blood-brain barrier depends on the unique characteristics of the brain capillaries.

Continuous tight junctions join the endothelial cells of brain capillaries. In addition, most brain capillaries are completely surrounded by a basement membrane and by the processes of supporting cells of the brain, called astrocytes.

The *BBB* permits passage of essential substances while excluding unwanted materials. Reverse transport systems remove materials from the brain. Large molecules such as proteins and peptides are largely excluded from crossing the blood-brain barrier. Acute cerebral lesions, such as trauma and infection, increase the permeability of the blood-brain barrier and alter brain concentrations of proteins, water, and electrolytes.

Some earlier animal experimental studies indicated that HBO increase the permeability of the cerebral vessels' walls in normal animals. *BBB* is disturbed in certain disorders such as cerebrovascular ischemia, and HBO may decrease the permeability of *BBB(Mink RB, 1995a)*

Cerebrospinal Fluid–Brain Barrier *(CSF)* & HBO effect on *(CSF)*

The ependymal cells covering the choroid plexus are linked together by tight junctions, forming a blood-CSF barrier to diffusion of many molecules from the blood plasma of choroid plexus capillaries to the CSF.

Water is transported through the choroid epithelial cells by osmosis. Oxygen and carbon dioxide move into the CSF by diffusion, resulting in partial pressures roughly equal to those of plasma. The high sodium and low potassium contents of the CSF are actively regulated and kept relatively constant. (Hollin SA, 1968) studied the effect of HBO on the oxygen tension of cerebrospinal fluid (CSF) and they found that CFS levels usually reflect the arterial pO2 tensions.

EFFECT OF HBO ON CEREBRAL BLOOD FLOW

Various studies were done regarding the cerebral blood flow, it generally recognized that oxygen has a vasoconstrictive action and reduces CBF, thought the mechanism is unknown. Extracellular superoxide dismutase plays a critical role in physiological response to oxygen in the brain by regulating nitric oxide (NO) availability (K.K.Jain, 2004). Recent studies have shown the implication of NO as a mediator of CNS oxygen toxicity. NO is formed from arginine by the action of one of three different nitric oxide synthesis (NOS), isozymes which are two calcium-dependent forms, (nNOS) and endothelial (eNOS), and one calcium-independent form (iNOS). The role of NO in epilepotgenesis was supported by two lines of evidence; (1) NO concentration in brain increase during exposure to HBO; (2) NOS inhibitors, which block NO production, can protect rats and mice against hyperoxic seizures (N. Bitterman, Bitterman, H.,, 1998; Chavsko, 1998; Thom, 2002)

According to the calcium-dependant NOS played a dominant role in the HBO-induced seizures (Mikulas Chavko, Xing, & Keyser, 2001)

There is a large conflict in the reports in this matter and arises from the variable effect of HBO on the normal versus the injured brain. If CBF is impaired by cerebral edema or raised ICP it can be improved by HBO.

(Jain, 2004; Leninger-Follert E, 1977) made an observation on the microcirculation and cortical oxygen pressure, during and after prolonged cerebral ischemia, that is relevant to the effect of HBO. According to these authors, complete cerebral ischemia of 1 h in cats followed by reactive hyperemia, and recirculation as well as reoxygenation of the brain can occur. Despite there would be a critical phase of a few hours after the recommencement of circulation as soon as reactive hyperemia ceases. If the brain swelling occurs can be prevented , however, the distribution of oxygen pressure in the cortex can be restored to normal, therefore HBO has a beneficial effect on cerebral edema.

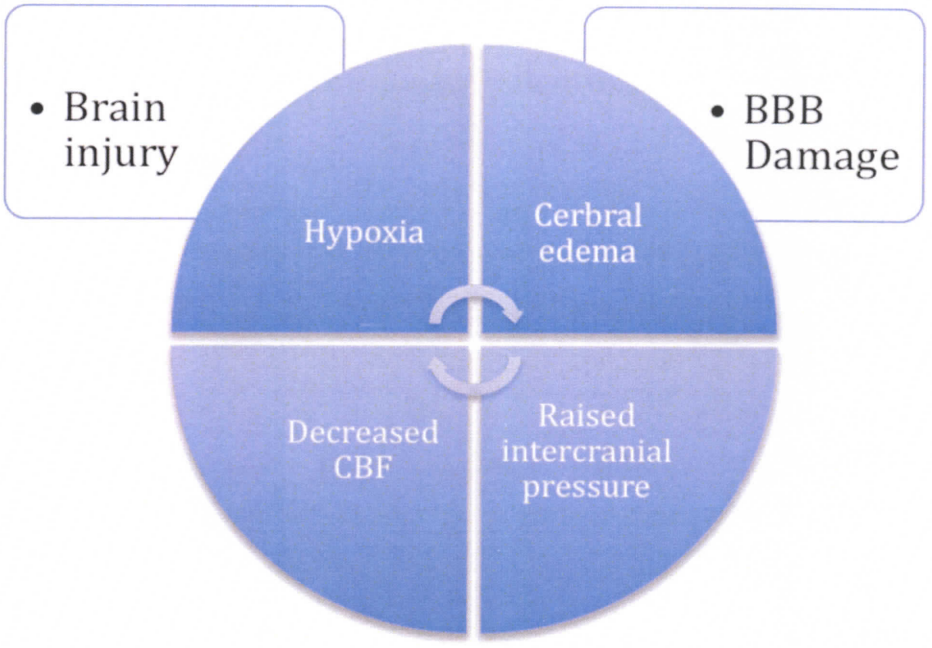

Figure 4: Hypoxia as a central factor for edema (Jain, 2004)

TYPES OF HYPERBARIC CHAMBERS AND GENERAL INDICATIONS OF THE HBOT

In hypoxia conditions, whether due to ischaemia or other factors, HBO reduces infection and cell death and maintains tissue viability while healing occurs. HBO is widely accepted as the only treatment for decompression sickness (DCS) and arterial gas embolism and the UHMS lists thirteen conditions for which'… research data and extensive positive clinical experience have become convincing (Gill & Bell, 2004)

There are several type of hyperbaric chambers, and the main concern is monoplace chamber which is a single chamber were the patient is laying on his back and the chamber it has several advantages main focuses is giving the patient a privacy and isolation of the patient in case of infectious diseases and the face mask is not required. Multiple chamber which could hold up to number of patients, the capacity is varies from a few to many as 20 patients the advantages of this chamber is simultaneous treatment of a large number of patients is possible, there is a reduced fire hazard, physical therapy can be performed during the treatment. Mobile chamber the first mobile chamber was constructed in the form of a bus in Nagoya, Japan but it is no longer in use. The advantages of this chamber that is portable which is easy to have and carry according to the situation in case of medical emergencies, it is comfortable and safe, it is an ideal for clinical use as well researchers, its suitable for military use (Gill & Bell, 2004; Jain, 2004).

Table 2: Types of chambers (Jain, 2004).

Type	Pressure	Type	Typical indications
I	Up to 1.5 ATA	Mobile and multiple	Ischemia disorders: cerebral, cardiac, peripheral-vascular: adjuvant to physical therapy and sport medicine; adjuvant to survival of skin flaps acoustic trauma
II	Up to 2.5 ATA Up to 3.0 ATA	Monoplace and portable	Gas gangrene, Burns, crush injuries of extremities Emergency treatment of decompression sickness
III	Up to 6.0 ATA	Multiplace chamber	Air embolism, Decompression sickness.

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Contraindications of hyperbaric medicine:

(Barnes, Bates, Cartlidge, French, & Shaw, 1985; Buras, Stahl, Svoboda, & Reenstra, 2000; Cabigas, et al., 2006; Chang, Niu, Hoffer, Wang, & Borlongan, 2000; Eric P. Kindwall, (September 3, 2004); Gill & Bell, 2004; Harch, Kriedt, Van Meter, & Sutherland, 2007; Jain, 2004; Kapp, Phillips, Markov, & Smith, 1982; Kudchodkar, Wilson, Lacko, & Dory, 2000; MCDOWALL, 1966; Mink & Dutka, 1995; Murata, Suzuki, Hasegawa, Nohara, & Kurachi, 2005; Nighoghossian & Trouillas, 1997; S. B. Rockswold, Rockswold, & Defillo, 2007; Rossignol & Rossignol, 2006; Rubinstein, et al., 2009; Sheridan & Shank, 1999)

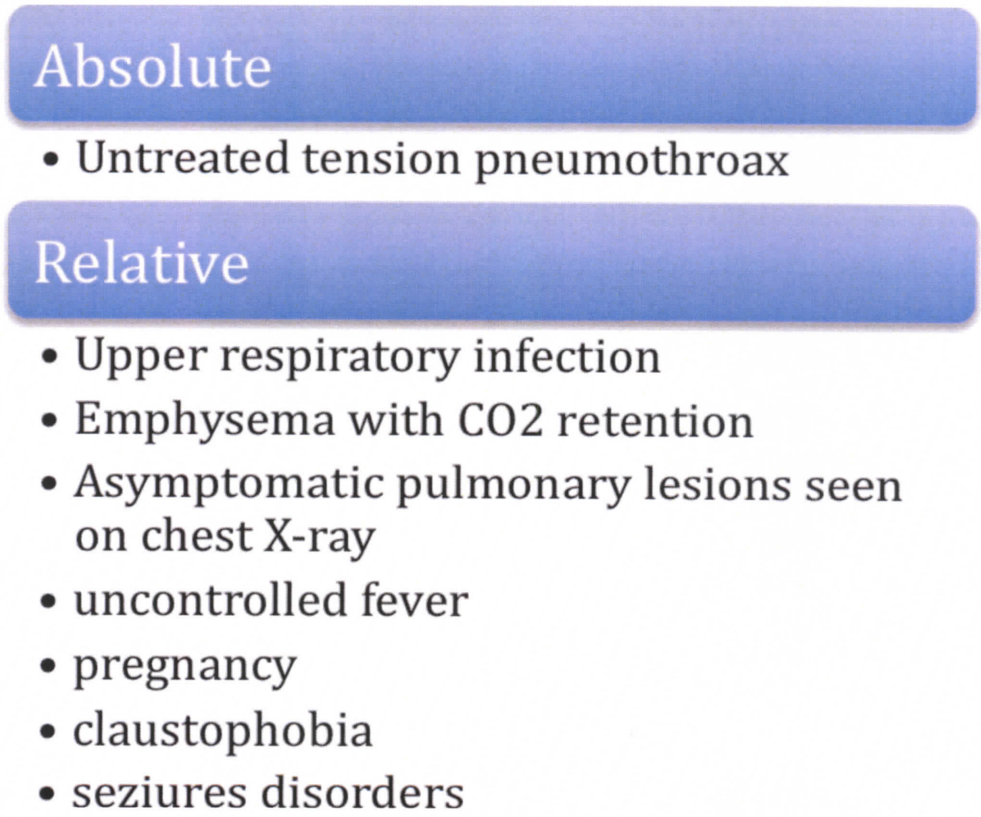

Figure 5 Absolut and retalive contraindications of HBOT

Uses of HBO Apporved by the Undersea and Hyperbaric Medical Society (UHMS):

- Air or gas Embolism
- Carbon monoxide, poisoning & carbon monoxide poisoning complicated by cyanide poisoning
- Clostridial myonecrosis (gas gangrene)
- crush injuries, the compartment syndrome and other acute traumatic ischemias
- Decompression sickness
- Enhancment of healing in selected problems wounds
- Exeptional anemia resulting from blood loss
- Necrotizing soft tissue infections (subcutaneous tissue, muscle, or fascia)
- Refractory osteomyelitis
- Radiation tissue damage (osteoradionecrosis)
- Compromised skin grafts and flaps
- Thermal burns

Figure 6: HBOT Indications by UHMS (Jain, 2004)

International indications of HBOT

- Decompression sickness
- Air embolism
- Poisoning: carbon monoxide, hydrogen, sulfide, carbon tetrachloride
- treatment of certain infections: gas gangrene, acute necrotizing fascitis, refractory mycoses, leprosy, osteomyelitis
- Plastic and reconstrective surgery: for nonhealing wounds surgery, as an aide to the survival of skin flaps with marginal circulation, as an aid to reimplantation surgery, as an adjucant to the treatment of burns
- Trumatology: crush injuries, compartment syndromes, soft tissue sports injuries
- Orthopedics: nonunion of fractures, bone grafts, osteoradionecrosis
- periperhal vascular diseases: ischemic gangrene, ischemic leg pain
- peripheral vascular diseases: shock, myocardial ischemia, aid to cardiac surgery
- Neurological: stroke, multiple sclerosis, migraine, cerebral edema, multi-infarct dementia, spinal cord injury and vascular diseases of spinal cord, brain abscess, peripheral neuropathy, radiation myelitis, vegetative coma
- Hematology: sickle cell crises, severe blood loss anemia
- Opthamology: occlusion of central artery of retina
- Gastor-interstinal: gastric ulcer, necrotizing enterocolitis, paralytic ileus, pneumotoides cystoides intestinalis, hepatitis
- for enchancement of radiosensitivity of malignant tumors
- otorhinolaryngology: sudden deafness, acute acoustic trauma, labyrinthitis, Meniere's disease, malignant otitis externa (chronic infection)
- Lung diseases: lung abscess, pulmonary embolism (adjunct to surgery)
- Obstetrics: complicated pregnancy, diabetes, eclampsia, heart disease, placental hypoxia, fetal hypoxia, congenital heart disease of the neonate
- Asphyxiation: drowning, near haning, smoke inhalation
- Aid to rehabilitation: spastic hemiplegia of stroke, paraplegia, chronic myocardial insufficiency, peripheral vascular disease.

Figure 7: International indications of HBOT (Jain, 2004)

OXYGEN TOXICITY

The pathology of oxygen toxicity has been documented comprehensively in a classical work on this topic by [Balentine 1982 (Jain, 2004)], it is well known that the development of pulmonary and CNS toxicity depends upon partial pressure and the duration of the exposure. Fortunately; the early effects of poisoning are completely reversible, but prolonged exposure first lengthens the recovery period and then eventually produces irreversible changes, many organs have been affected in experimental oxygen toxicity studies of long exposure to high pressures, a situation that is not seen in clinical practice (Richard A. Neubauer, 2001)

Pulmonary oxygen toxicity is usually manifestation of prolonged exposure (more than 24 hour) to normobaric 100% oxygen, as well as during exposure to HBO from 2 to 3 ATA O2 in human and experimental animals. The major mechanism by which HBO produces lung injury in rabbits is by stimulating thromboxane synthesis according to (Jacobson et al 1992, (Jain, 2004); Lung injury induced by free radicals has been demonstrated in an animal model of smoke inhalation, and the free radicals clear up after about an hour [Yamagauchi et al 1992 (Jain, 2004)] Normobaric 100% of oxygen given for one hour dose not increase the level of free radicals in this model , but HBO at 2.5 ATA dose so (Richard A. Neubauer, 2001)

Further more this is some summarized clinical trails and studies, which failed due to oxygen toxicity:

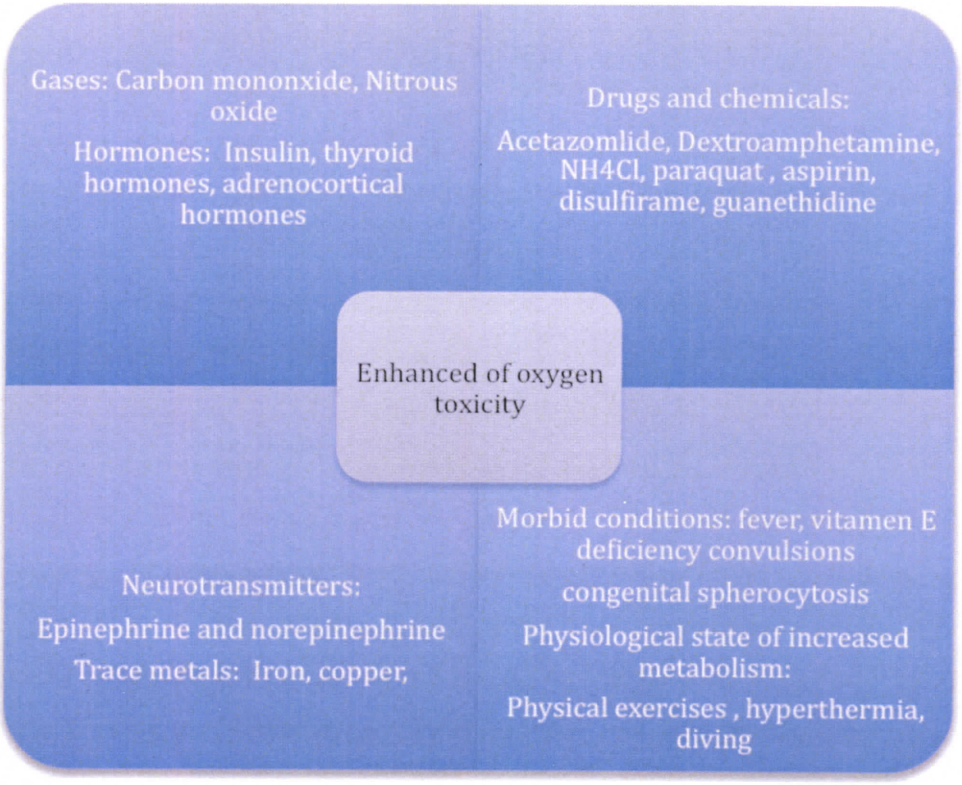

Figure 8: Enhance of oxygen toxicity (Jain, 2004)

HBO IN NEUROLOGICAL DISORDERS

Various neurological conditions where HBOT has been reported to be useful, there are few controlled clinical studies, the UHMS USA dose not list any of these conditions (with exceptional to cerebral air embolism) as approved for payment by third-party insurance carriers.

There are several good reviews of the use of HBO in neurological disorders we will be adding the indications in neurological disorders briefly in this

Table 3: HBOT in Neurological disorders (Barnes, et al., 1985; Cabigas, et al., 2006; Chang, et al., 2000; Gill & Bell, 2004; Harch, et al., 2007; Hayakawa, Kanai, Kuroda, Yamada, & Mogami, 1971; Jain, 2004; Kapp, et al., 1982; Koch & Vermeulen-Cranch, 1962; Kudchodkar, et al., 2000; MCDOWALL, 1966; Nighoghossian & Trouillas, 1997; S. B. Rockswold, et al., 2007; Rossignol & Rossignol, 2006; Rubinstein, et al., 2009; Sheridan & Shank, 1999; Zhang, Lo, Mychaskiw, & Colohan, 2005)

1. Cerebrovascular disease: *Acute cerebrovascular occlusive disease, Chronic post-stroke stage, treatment of spasticity, aid to rehabilitation, adjunct to cerebrovascular surgery, selection of patients for IC/EC by pass operations on the basis of response to HBO, Post-operative complications of intracranial aneurysm surgery: (Cerebral edema and raised intracranial pressure)*
2. Cerebral air embolism
3. Head injuries: *Cerebral edema raised intracranial pressure*
4. Spinal cord lesions

Acute traumatic paraplegia within 4 hours of injury, spinal cord decompression sickness (Spinal cord "hit"), ischemic diseases of the spinal cord, aid to the rehabilitation of paraplegia and quadriplegia, residual neurological deficits after surgery of compressive spinal lesion

5. Cranial nerve lesion: *Occlusion of the central artery of the retina, facial palsy, sudden deafness, vestibular disorders*
6. Peripheral neuropathies
7. Multiple sclerosis
8. Cerebral insufficiency (Decline of mental function): *multi infarct dementia*

9. Infection of the CNS and its coverings: *brain abscess, meningitis*
10. Radiation- induced necrosis of the CNS: *radiation myelopathy, and encephalopathy*
11. CO poisoning
12. Migraine headaches
13. Cerebral palsy
14. Autism (*A new hypothesis & approach still understudies)* (Yildiz, Aktas, & Uzun, 2008); the author didn't suggest the usage but some other articles recommended it usage.

Scientific researchers world wide have been researching experimenting the hyperbaric medicine in different medical cases, there were a big debate on the use of the HBOT and the main concern were the base line to recommend indicate the HBOT or to contraindicate the usage of this method specially in the relation to the production of free radicals.

DNA DAMAGE, FREE RADICALS, OXYGEN TOXICITY IN HBOT

[Dennog and colleagues (C. Dennog, A. Hartmann, G. Frey, & G. Speit, 1996)] they experiment on 10 healthy male volunteers (non-smoker, aged 25-34 years) gave informed consent to participate in this study they were exposed to 100% O2 at 2.5 ATA, they were given a 3x20 min, interspersed with 5 min periods of air breathing. With this study we demonstrated for the first time the induction of oxidative DNA damage by HBO. A clear reproducible genotoxic effect was seen with the comet assay immediately after HBO exposure (3x20 min at 2.5 ATA), as used in therapeutically.

Another study was done by [Speit and colleagues (Speit, Dennog, Eichhorn, Rothfuss, & Kaina, 2000)]; they tested fourteen healthy volunteers (non-smoker, aged 25-34 years) gave informed consent to participate in this study, the research was approved by university human subjects committee. They were exposed to 100% O2 at a pressure 2.5 ATA

in HBO for a total of three 20 min periods, interspersed with 5 min periods of air breathing (standard protocols), or for one or two 20 min periods, venous blood were taken before HBO, immediately on exit from the chamber 1 day later. The study shown that treatment of individuals with a single standard HBO induces adaptation of lymphocytes against the genotoxic effects of an oxidative agents such as H2O2, they looked for molecular adaptive response adaptation can be caused by increase in the level of DNA repair functions or enhancement of general defense against oxidative reactive species.

[Demurov and colleagues (Demurov, Mil'chakov, Bogdanova, Koloskov Iu, & Tepliakov, 1986)] studied the Daily exposure of rabbits to HBO at 2.5 ATA (in 1-h sessions) for 28 days has been shown to reduce the activity of antioxidant enzymes and tissue resistance to lipid per oxidation, one of the consequences was impairment of the cardiac contractile function, and necrotic foci were observed in the myocardium. A high-pressure oxygen leads to increased pyruvate/lactate, and pyruvate malate redox couples, as well as to a decrease in the incorporation of phospholipid long chain-fatty acid and pyruvate into the tissue lipid. During recovery from the effect of high-pressure oxygen these changes are reversed. These data indicate that oxygen poisoning of tissue is not the result of an inhibition of carbohydrate metabolism, but instead may result from the formation of toxic lipoprotein.

Pulmonary oxygen toxicity is a usual manifestation of prolonged exposure to normobaric 100% oxygen, as well as during exposure to HBO from 2 to 3 ATA O2 in human and experimental animals (Jain, 2004)

The side effects of pressured oxygen therapy have been noted, especially for high pressure (>3 ATA) and long duration. It has been shown that HBO (4.96 ATA for 1 h) administered to rats can cause CNS toxicity. A previous study has shown that exposing rats to 4ATAat 100% oxygen for 90 min was associated with an increased level of lipid peroxidation product, and altered enzymatic anti-oxidation (glutathione peroxidase) in the brain. High levels of HBO reduced cerebral blood flow, possibly by reducing nitric oxide syntheses [Chavko et al.(Jain, 2004)] stated that 100% O2 at 5 ATA

induced seizures. Long duration of HBO at high-pressure levels results in adverse effects due to the onset of oxygen toxicity as manifested by the induction of lipid peroxidation and seizures. (Zhang, et al., 2005)

MECHANISMS OF HYPERBARIC OXYGEN AND NEUROPROTECTION IN STROKE

Scientific basis

The most important factor determining the effectiveness of HBO in ischemic stroke is the therapeutic window for treatment. The primary supposition of HBO therapy in stroke treatment is not to treat the ischemic core but the surrounded areas that are viable yet non-functioning, where residual viable neuronal cells might be salvaged. Therefore, the therapeutic window for intervention with HBO should be similar to that for thrombolytic therapy, with a window of 3–6 h when the ischemic neuronal tissues can still be saved (Zhang, et al., 2005).

Historically, the mechanism through which HBO worked was found to be vasoconstriction of the cerebral blood vessels, which led to decreased CBF and ICP. The vasoconstriction was not found to be deleterious because O2 availability to the injured cells was greatly increased (S. B. Rockswold, et al., 2007).

HBO has an important role in improving the microcirculatory disturbances, the resistance to blood flow is greater in vessels in which the diameters is less than 1.5 mm, e.g. the capillaries. The viscous effect is less, because red blood cells (RBC) are aligned in a column as they pass through lumen *(Farhraeus Lindqvist effect),* instead of moving randomly. In microcirculatory disturbances, such as occur with clumping of RBC and slowing of circulation, the viscosity of the blood increases tremendously. RBC can get stuck where the endothelial cells protrude into the lumen of the capillary. The blood flow can become totally blocked for a fraction of a second or a longer period. Some plasma may, however. Seep across the obstruction.

HBO has a beneficial effect in cerebral ischemia through the following mechanism (Jain, 2004):

1. Oxygen dissolved in plasma under pressure raises pAO2 and can nourish the tissues even in the absence of RBC.
2. Oxygen can diffuse extravascularly. Diffusion is facilitated by the gradient between high oxygen tension in the patent capillaries and the low tension in the occluded ones, the effectiveness of this mechanism depends upon the abundance of capillaries in the tissues', because the brain is a very vascular tissue, this mechanism can provide for the oxygenation of the tissue after vascular occlusive.
3. Decreasing the viscosity of the blood and reducing platelet aggregation and increasing RBC deformability can facilitate the supply of oxygen to the tissue.
4. HBO relives brain edema, by its vasoconstricting action, HBO counteracts the vasodilatation of capillaries in the hypoxic tissue and reduces the extravasations' of fluid.
5. HBO also reduces the swelling of the neurons by improving their metabolism.
6. HBO, by improving oxygenation of the penumbra that surrounds the area of total ischemia, prevents glycolysis and subsequent intracellular lactic acidosis and maintains cerebral metabolism in an otherwise compromised area.

RATIONALE USE OF HYPERBARIC OXYGNATION

Breathing atmospheric air at sea level, 0.3 ml of oxygen are dissolved in each 100 ml of plasma, the remaining 19.1 ml of oxygen being transported as oxyhaemoglobin. This latter figure cannot be increased once the hemoglobin is fully saturated. At 2 ATA of pure oxygen 4.3 ml of oxygen are carried dissolved in each 100 ml of blood, this representing a fourteen-fold increase in the increased dissolved oxygen. The relative importance of

these two conflicting factors (the increased dissolved oxygen and the reduce tissue flow) is uncertain. Experimental work in animals suggested that the net effect of HBO might be harmful. However [Ashfield and Gavey 1969] showed that the effect was beneficial in man (Thurston, Greenwood, Bending, Connor, & Curwen, 1973)

REVIEW OF CLINICAL STUDIES

Animals experiments

No animal model can mimic the human stroke and there are fallacies of testing neuroprotective compounds in animal models, some of these results are difficult to translate into human clinical trails. One advantage, however; of these models is that standard model can be used to compare various therapies. Stroke models may be quite appropriate for evaluating the effects of HBO in acute cerebral infarction.

Animal experimental evidence for the effect of HBO well be summarized in this following table (Jain, 2004; Richard A. Neubauer, 2001, February 7, 2001). *(See table at the appendix 1)*

Uncontrolled group

Most of the studies of HBOT in acute stroke are uncontrolled. Only a few controlled studies have been done in recent years and further studies are planned. Considerable background information can be extracted from the uncontrolled studies reported in the literature.

Various studies on the clinical application of HBO in cerebrovascular diseases, as reported in the following table (Jain, 2004; Richard A. Neubauer, February 7, 2001), the total report cases were over 1000. The reported rate of improvement were 40% to 100% and is much higher than the natural rate of recovery, particularly because many of the reported cases were in a chronic post stroke stage with stable neurological deficits the major criticism in these studies that none of them were a randomized control study. *(See the table at the appendix 2)*

Controlled clinical trails

Such trails are considered necessary because of the value of HBO therapy in acute stroke remains controversial. Controlled trails will be required for proving the efficacy of HBO in acute stroke because it is difficult to prove that the recovery is due to treatment and natural recovery. The other issue is demonstrating the safety of HBO in acute stroke patients, for number of logistic problems, it has not been easy to carry out clinical trails of HBO in acute stroke. *(See the table at the appendix 3)*

[Anderson and colleagues', (D. C. Anderson, et al., 1991; Nighoghossian, Trouillas, Adeleine, & Salord, 1995)] administrated HBO in a double blind prospective protocol to 39 patients with acute ischemic cerebral infarction. They aborted the study when no dramatic improvement was notes in the HBO treated patients. Rather, there was a favoring the control patient (treated with only air) who has less severe neurological deficits and smaller infarcts. This trend was considered to be an artifact of randomization process. The treatment protocol was broken in 19 of 39 patients and 8 patients refused to continue treatment. The results of this trail neither prove or disapprove the usefulness of HBO in acute stroke and can be disregarded. They merely point out the difficulties in carrying out such a trails.

(Nighoghossian & Trouillas, 1997; Nighoghossian, et al., 1995) reported the result of a randomized trail only on 34 patients. These were enrolled over a period of three years. Half of these received HBO whereas the other halves were treated in hyperbaric air. There was no significant difference at inclusion between the two groups regarding age, time of stroke onset to randomization, and Orgogozo scale. The mean score of HBO group on the Rankin score. There authors concluded that HBO was safe in these patients and there was an outcome trend favoring HBO therapy. They recommended a large-scale controlled trails. In recent review these, authors recommend a large double-blind trail of HBO, possibly combination with thrombolytic therapy.

(Rusyniak, et al., 2003) conducted a randomized, prospective, double blind, sham-controlled pilot study of 33 patients presenting with acute ischemic stork who did not receive thrombolytic over a 24-month period. Patients were randomized to treatment for 60 min in a monoplace hyperbaric pressurized with 100% O2 at 2.5 ATA in the HBO group or 1.14 ATA in sham group. Primary outcomes measured included percentage of patients with improvement at 24 h using NIHSS scale *(National Institutes of Neurological Disorders and Stroke)* , and 90 days (NIHSS, Barthel Index, modified Rankin Scale, Glasgow Out come Scale), secondary measurements included complications of treatment and mortality at 90 days. Results revealed no differences between the groups at 24 h. At 3 months, however, a larger percentage of the sham patients had a good outcomes defined by their stroke scores compared with HBO group (NIHSS, 80% versus 31.3; P=0.004; Barthel Index, 81,8% versus 50% P=0.12; modified Rankin Scale, 81.8% versus 50%; P= 0.02; Glasgow Outcome Scale, 90% versus 37.5%; P=0.01) with loss statistical significance in a intenet-to-treat analysis. It was concluded that HBO dose not appear to be beneficial and in face may be harmful in patients with acute ischemic stroke, the design of this study, however. Invalidate any conclusions because of HBO at 1.5 ATA has been established to be a safe pressure to treat patients with acute stroke. The control group was closer to the parameter.

Random studies with HBOT treatment *(see the table in appendix 4)*

3.2.2 Oxygen radicals and the disease processing "Oxidative Stress" & "Free Radicals"

Disruption of oxygen and glucose supply results in abnormal cellular metabolisms and various molecular, cellular, and physiological responses in cells in affected brain regions. Experimental evidence has suggested that abnormal free radical metabolism contributes, at least in part, to the damage that occurs after brain ischemia.(H. Shi & Liu, 2007).

A free radical defined as; any species capable of independent existence that contains one or more unpaired electrons. (e.g. of free radicals are Superoxide (O2) and hydroxyl (OH) both oxygen-centered radicals, most biological molecules are non-radical containing only paired electrons, when non-radicals and radicals react together free radical chain reaction often result (Slater, 1984; Thomas, 1998; Wu & Cederbaum, 2003).

Free radical consists of two categories: reactive oxygen species (ROS) and reactive nitrogen species (RNS). One of the major RNS in cerebral ischemia is nitric oxide (NO), a water and lipid soluble free radical, by the action of nitric oxide syntheses (NOS). There are three isoforms of NOS in brain cells, neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). Neurons produce NO mostly from nNOS activation. Glial cells generate NO mainly from iNOS activation (H. Shi & Liu, 2007).

The molecular basis of CNS oxygen toxicity, and also pulmonary oxygen poisoning, involves generation of reactive oxygen species, (ROS). This is has been known as the free radical theory of oxygen poisoning. (Jain, 2004) The presences of free radicals in biological materials was discovered less than 50 years ago (Scheibmeir, et al., 2005).

Oxygen free radicals are normally produced by the mitochondria during electron transport, and, after ischemia, high levels of intracellular Ca2+, Na+, and ADP stimulate excessive mitochondrial oxygen radical production.(Aneesh B. Singhal, 2006). Free radicals play a key role in both normal biological function and in the pathogenesis of many disease processes. These free radicals are continuously formed in the body and are

exquisitely balanced. When this balance is broken, oxidative stress occurs. During oxidative stress, cellular lipids, DNA, and proteins are attacked causing damage to the cells and resulting in a loss of function, integrity, and ultimately, cellular death (Whitaker & Pierce, 2003).Today there is a large evidence indicating that patients are exposed to excessive free radicals, from drugs and other substances that alert cellular reduction-oxidation (Redox) balance, and disrupt normal biological function (Scheibmeir, et al., 2005). Most chemical reactions in the body are "balanced" through reduction and oxidation mechanisms (redox). Redox reactions primarily involve the transfer of electrons between two chemical species(Whitaker & Pierce, 2003). The compound that loses an electron is said to be oxidized; the one that gains an electron is said to be reduced. A free radical may be defined as any species that has one or more unpaired electrons. This broad definition includes the hydrogen atom (one unpaired electron), most transition metals and the oxygen molecule itself. O2 has two unpaired electrons, each located in a different, orbit anti-bonding orbital, these two electrons have the same spin quantum number and so if O2 attempts to oxidize another atom or molecule by accepting a pair of electors from it, both new electrons muse be parallel spin so as to fit into the vacant spaces in the orbital's (Halliwell & Gutteridge, 1984).

Most molecules can become free radicals by either losing or gaining an electron. During this chemical process, molecules can be either reduced or oxidized. A single electron in an orbital is said to be "unpaired". Thus, a radical is defined as any species that contains one or more unpaired electrons capable of independent existence. When electrons are paired, the molecule is chemically stable in contrast to a molecule with an unpaired electron. An orbital that has only one electron will try to fill it by "stealing" another unpaired electron from the orbital of another molecule. The donation of an electron is called oxidation and the gain of an electron is called reduction (Whitaker & Pierce, 2003).

The most common ROS include: the superoxide anion (O2-), the hydroxyl radical (OH ·), singlet oxygen (1O2), and hydrogen peroxide (H2O2)

Superoxide anions are formed when oxygen (O2) acquires an additional electron, leaving the molecule with only one unpaired electron. Within the mitochondria O2- · is continuously being formed. The rate of formation depends on the amount of oxygen flowing through the mitochondria at any given time. Hydroxyl radicals are short-lived, but the most damaging radicals within the body. This type of free radical can be formed from O2- and H2O2 via the Harber-Weiss reaction. The interaction of copper or iron and H2O2 also produce OH · as first observed by Fenton. These reactions are significant as the substrates are found within the body and could easily interact (Sandeep Raha, 2001; Thomas, 1998; Zhou, et al., 2007).

Most chemical reactions in the body are "balanced" through reduction and oxidation mechanisms (redox), redox reactions primarily involve the transfer of electrons between two chemical species. It is important to remember that the human body attempts to maintain a balance between the reactive oxygen species production and the levels of antioxidants. This balance is often referred to as the "redox potential" and interference of the balance may lead to cellular damage. Changing balance towards an increasing in oxidants is called "oxidative stress", and changing the balance towards an increase in the redacting power, or antioxidants, is called reductive stress (Whitaker & Pierce, 2003). Reactive oxygen species (ROS) are small, highly reactive, oxygen–containing molecules that are naturally generated in small amounts during the body's metabolic reactions and can react with and damage complex cellular molecules such as fats, proteins, or DNA. Alcohol promotes the generation of ROS and/or interferes with the body's normal defense mechanisms against these compounds through numerous processes, particularly in the liver. For example, alcohol breakdown in the liver results in the formation of molecules whose further metabolism in the cell leads to ROS production. Alcohol also stimulates the activity of enzymes called cytochrome P450s, which contribute to ROS production (Wu & Cederbaum, 2003).

Oxidative stress: the term is used in the literature, but rarely defined, in essence it referees to the situation of a serious imbalance between produxtion of ROS/RNS and antioxidant defense. Sies introduced a somewhat-vagye definition in 1992, as a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage. In principle oxidative stress can result from(Richard A. Neubauer, 2001; Sies, 1997):

1. Diminished antioxidant, e.g. mutations affecting antioxidant defense enzymes.
2. Increased production of ROS/RNS e.g. by exposure to elevated O2, the presence of toxins that are themselves reactive species (e.g. NO) metabolized to generate ROS/RNS, or excessive activation of "natural" ROS/RNS producting system (e.g. inappropriate activation of phagocytic cells in chronic inflammatory disease such as rheumatoid arthritis and ulcerative colitis),

Oxidative stress can result adaptation: by up regulating of defense system which may(Thomas, 1998):

- Completely protect against damage
- Protect against damage but not completely
- "Over protect", e.g. the cell is then resistant to higher levels of oxidative stress imposed subsequently.

Metabolic activation and cell injury

Many substances are metabolically activated to a free radical intermediate through interactions with the NADPH-cytochrome P-450electrontransport chain, which is located in the endoplasmic reticulum in many types of cell and involves the flavoprotein NADPH-P-450 reductase and cytochrome P-450 together in a phospholipid environment. Although P-450 is mainly located in liver, small amounts of P-450 can be detected in many other tissues (Slater, 1984).

INFLAMMATION IN RELATION WITH FREE RADICALS AND THE BBB TOXICITY

Considerable attention has been directed to a possible involvement of free radical intermediates in the inflammatory process; in particular, to the beneficial effects of superoxide dismutase that acts to decrease the concentration of 02- generated by local metabolic disturbances and by stimulated white cells attracted to the site by chemotactic agents. Although (Jain, 2004)form, O2H', is more reactive and in the presence of transitional metal ions 02- can generate OH which would contribute to the many toxic effects associated with superoxide production in biological systems. The phenomenon overall is complex and multiphasic, but an associated key process is the so-called 'metabolic burst' during which reduced oxygen species are produced. Free radical intermediates have been linked recently to a number of clinically important disturbances including ischaemic attacks (Slater, 1984).

The toxicity of the abnormal free radical metabolism in cerebral ischemia/reperfusion results from their modification of macromolecules, from their effect on signal transduction pathways, and from the resulting induction of apoptotic and necrotic pathways. Their involvement in blood brain barrier (BBB) disruption, apoptosis, and cellular response to hypoxia are discussed as follows (Richard A. Neubauer, 2001)

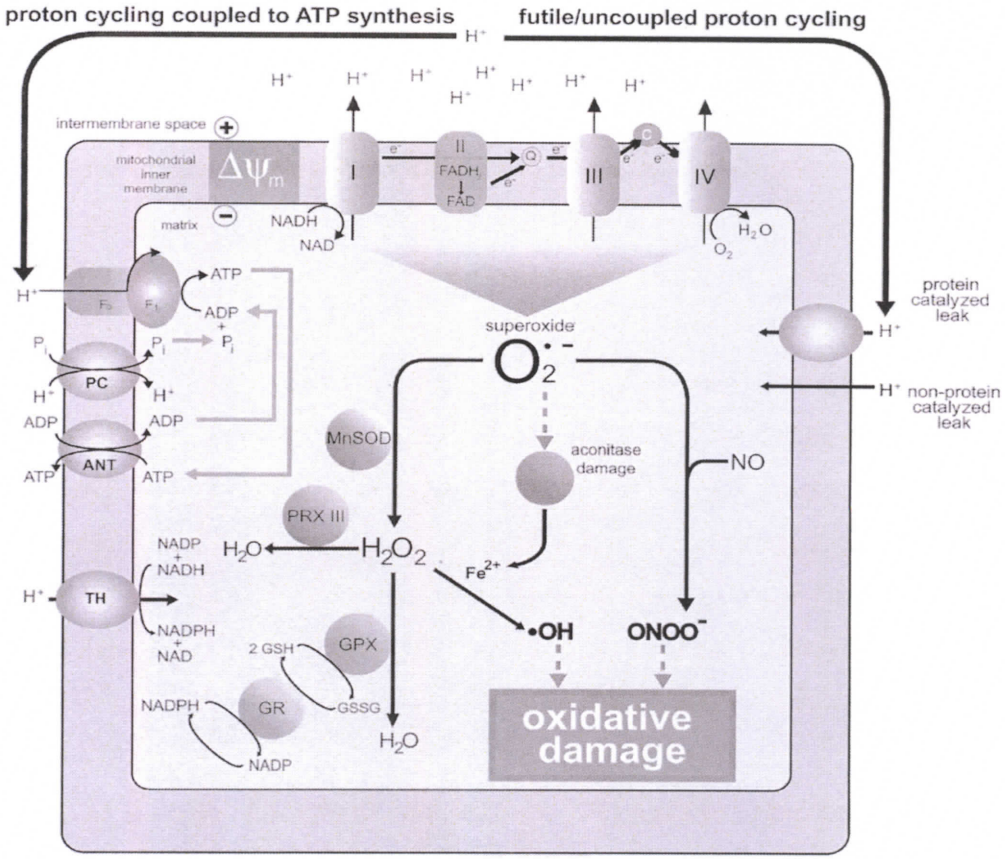

Figure 9: Mitochondrial oxidative stress (Green, C., nbsp, & Reed, 1998; K. Green, Brand, & Murphy, 2004)

Legend

Mitochondrial oxidative damage. The mitochondrial respiratory chain (top) passes electrons from the electron carriers NADH and FADH2 through the respiratory chain to oxygen. This leads to the pumping of protons across the mitochondrial inner membrane to establish a protonelectrochemical potential gradient (H), negative inside: only the membrane potential (m) component of H is shown. The H is usedto drive ATP synthesis by the F0F1ATP syntheses. The exchange of ATP and ADP across the inner membrane is catalyzed by the adenine nucleotide transporter (ANT) and the movement of inorganic phosphate (Pi) is catalyzed by the phosphate carrier (PC) (top left). There are also proton leak pathways that dissipate H without formation of ATP (top right). The respiratory chain also produces superoxide (O2), which can react with and damage iron sulfur proteins such as aconitase, thereby ejecting ferrous iron. Superoxide also reacts with nitric oxide (NO) to form peroxynitrite (ONOO). In the presence of ferrous iron, hydrogen peroxide forms the very reactive hydroxyl radical (OH). Both peroxynitrite and hydroxyl radical can cause extensive oxidative damage (bottom right). The defenses against oxidative damage (bottom left) include MnSOD, and the hydrogen peroxide it produces is degraded by glutathione peroxidase (GPX) and peroxiredoxin III (PRX III). Glutathione (GSH) is regenerated from glutathione disulfide (GSSG) by the action of glutathione reductase (GR), and the NADPH for this is in part supplied by a
transhydrogenase (TH). (K. Green, et al., 2004)

THE GENERATION OF OXYGEN FREE RADICALS BY MITOCHONDRIA

Life on earth envolved in changing milieu with respect to oxygen, micro-organisms that had arisen in a reducing environment, in adapting to increasing atmospheric level of oxygen, faced a new problem-that of reactive oxygen species (ROS), produced as a byproduct of normal metabolism. (Raha & Robinson, 2000)

The oxygen molecule is capable of accepting an additional electron to create superoxide, a more reactive form of oxygen.

The most oxidinized radical that is likely to arise in a biological system is the Hydroxyl radical HO°, being at the top order of it can oxidizien all the reduced species below it. (Buettner, 1993)

Equation 1: Fe(II) complex: (Buettner, 1993)

$$Fe(II)complex + H_2O_2 \rightarrow Fe(III)complex + OH + HO^{\bullet}$$

Equation 2: Superoxide (Raha & Robinson, 2000)

$$O2 + 1e \rightarrow O2-$$

Superoxide is the primary oxygen free radical produced by mitochondria and is probably produced by a non-enzymic mechanism. Ubisemiquinone generated in the course of electron transport reactions in the respiratory chain donates electrons to oxygen and provides a constant source of superoxide (Sandeep Raha, 2001)

Equation 3: Superoxide on sulfur (Raha & Robinson, 2000)

$$UQ10 + O2 = O2- + UQ10$$

Superoxide can attack iron sulfur centers in enzymes such as aconitase, succinate dehydrogenase, and mitochondrial NADH-ubiquinone oxido reductase, releasing iron and destroying catalytic function. Superoxide is, therefore, rapidly removed by conversion to hydrogen peroxide in a

reaction catalysed by superoxide dismutases. (Raha & Robinson, 2000; Sandeep Raha, 2001)

Superoxide its self can be toxic, especially through inactivation of proteins that contain iron-sulphur centers such as aconitase and succinate. Dehydrogenase and mitochondrial N-methyl-D- aspartate (NADH)-ubiquinone oxidoreductase, Ferrus iron released during the inactivation becomes an important reactant in the fenton reaction(Raha & Robinson, 2000)

Equation 4: H2O2 + Fe++

$$H2O2 + Fe{+}{+} = OH^- + OH' + Fe{+}{+}{+}$$

Metabolism strips electrons from fatty acids, sugars, and amino acids and accumulates them on the soluble electron carrier NADH and on protein-bound FADH2, the electrons are then passed down the mitochondrial respiratory chain to drive ATP synthesis by oxidative physphorelation. As the electrons move down the potential energy gradiant from NADH/FADH2 to oxygen, the redox energy is conserved by pumping proton electrochemical potential gradient (K. Green, et al., 2004)

Complex I and superoxide production

Oxidative phosphorylation in mitochondria is carried out by four electron-transporting complexes (I-IV) and one H+ translocating ATP synthetic complex (complex V). Two of these complexes were shown to be responsible for much of the superoxide generated by mitochondria: complex I, the NADH- ubiquinone oxidoreductase, and complex III, the ubiquinone-cytochrome c oxidoreductase. (Raha & Robinson, 2000)

Damage caused by mitochondrially generated ROS

Under normal circumstances, the rate of generation of superoxide from mitochondria is rather low and dose little damage, simply because it is efficiently removed by the superoxide dismutase's.Circumstances can arise for a variety of reasons (e.g. ingested chemicals that act as radical

amplifiers, medically applied high concentrations of oxygen, or during periods of reperfusion of tissue with oxygen following ischemia). Where high rates of superoxide production do occur, superoxide itself is especially damaging to the (4Fe-4S) type of iron-sulphur centre. Damage of isoenxyme of aconitase evident after exposure to superoxide results in the release of ferrous ions and also sets in motion an important regulators mechanism: the deactivated enzyme becomes a binding protein for the mRNA for ferritin, prolonging its half-life and ensuring increased ferritin synthesis to bind free iron. The damage to other systems such as mitochondrial aconitase complex succinate and I dehydrogenase, Damage of these enzymes by superoxide undoubtedly impairs the normal functioning of the citric acid cycle. This combined with resulting inactivation of electron transport processes. Then result in disordered metabolism with lack of mitochondrial ATP generation, leading to increase flux from glucose to lactate (Raha & Robinson, 2000).

Mitochondrial ROS production in vivo

The mitochondrial ROS production occurs at all time is suggested by mice lacking MnDOS, which dise within a few days of birth, which those lacking the cytosolic isoform Cu,ZnSOD survive, further evidence of mitochondrial ROS production under normal conditions is the efflux of hydrogen peroxide from intact mitochondria produce superoxide, which is then converted to hydrogen peroxide in vivo. There is also evidence that, under certain conditions, mitochondrial DNA and protein accumulate greater oxidative damage in vivo than the rest of the cell. Many other enzymes associated with mitochondria can also produce superoxide or hydrogen peroxide, but even through their contribution to ROS formation in vivo is unclear (K. Green, et al., 2004).

Superoxide Production From the Respiratory Chain

The mitochondrial respiratory chain is the major site of ROS production within the cell, Superoxide is thought to be produced continually as a byproduct of normal respiration through the one electron reduction of molecular oxygen, superoxide itself damages iron sulfur-center containing enzymes such as aconitase, and can also react with nitric oxide to form the damaging oxidant peroxynitrite which is more reactive than either precursor.

The mitochondrial respiratory chain is made up of four electron-transporting complexes (I-IV) and one proton translocating ATP synthetic complex (complex V) Two of these complexes were shown to be responsible for much of the superoxide generated by mitochondria: complex I, the NADH-ubiquinone- oxidoreductase, and complex III, the ubiquinol cytochrome c oxidoreductase.(Sandeep Raha, 2001)

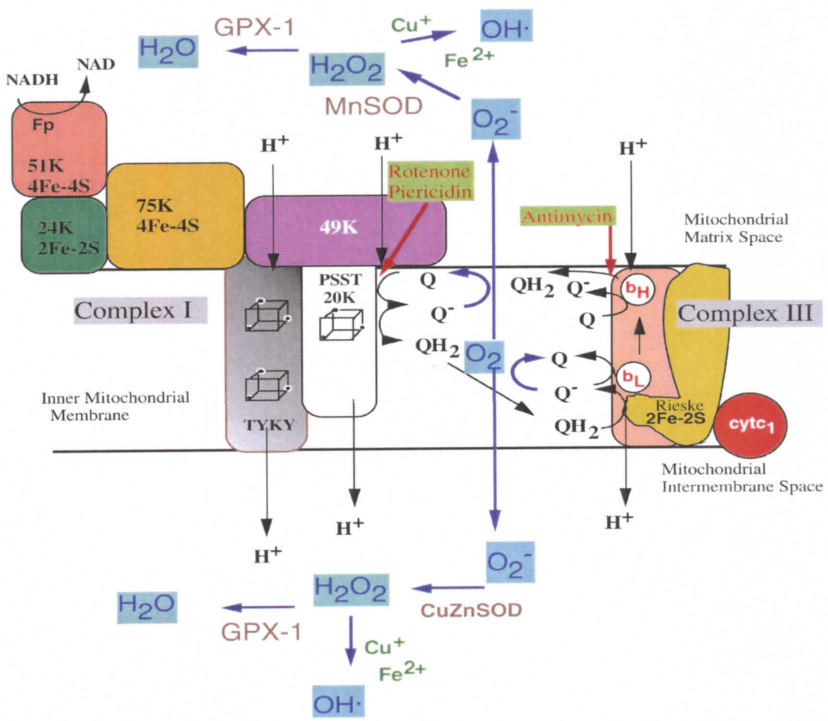

Figure 10: Relation between respiratory chain components of Complex I (Raha & Robinson, 2000)

Legend:
Diagram to show the relationship between the respiratory chain components of complex I and III and the site of superoxide production. The oxidation of NADH generated intramitochondrially is achieved by the 51 kDa (K) ¯avoprotein containing subunit of complex I which then transfers electrons to its own iron sulfur center. Subsequently, electron transfer through the 24 kDa and 75 kDa iron sulfur proteins to the TYKY 23 kDa and finally the PSST 20 kDa iron sulfur proteins embedded partly in the inner mitochondrial membrane. Final reduction of ubiquinone to ubisemiquinone and ubiquinol takes place at one, or possibly two, sites defined by the presence of the 49 kDa and PSST proteins which have evidence of quinone binding capacity. In complex III, the Rieske iron sulfur protein to produce ubisemiquinone, which then is oxidized to quinone by the bL heme of cytochrome b, oxidizes ubiquinol. The electrons are passed to the bH heme, which then re-reduces the ubiquinone to semiquinone and ubiquinol at the antimycin A sensitive site close to the mitochondrial matrix face of the membrane. Cyclic ¯own of electrons and reducing equivalents between cytochrome b and quinone coupled with proton movement and passage of electrons through to cytochrome c1 and cytochrome c completes the series of transfers. Superoxide can be generated in at least two places where ubisemiquinones are produced by simple chemical reaction with molecular oxygen. The superoxide produced can be transformed into hydrogen peroxide by either manganese superoxide dismutase or the copper/ zinc superoxide dismutase, depending on the location. Hydrogen peroxide has two fates. Either it is reduced to water by glutathione peroxidase, or is converted into the damaging hydroxyl radical if free cuprous or ferrous ions are available (Sandeep Raha, 2001).

OXIDATION & DAMAGE IN DNA, PROTEIN & LIPIDS ,IN RELATION TO AGING PROCESS, CANCER, BRAIN INJURY

The degenerative diseases associated with aging include cancer, cardiovascular disease, immune system decline, brain dysfunction, and cataracts, the functional degeneration of somatic cells during aging appears, in contribute with these diseases (Ames, Shigenaga, & Hagen, 1993)

Extensive studies with model systems, and with biological materials in vitro, have clearly shown that reactive free radicals are able to produce chemical modifications of, and damage to, proteins, lipids, carbohydrates and nucleotides. Therefore, if such reactive free radicals are produced in vivo, or in cells in vitro, in amounts sufficient to overcome the normally efficient protective mechanisms, we can expect metabolic and cellular disturbances to occurs (Slater, 1984)

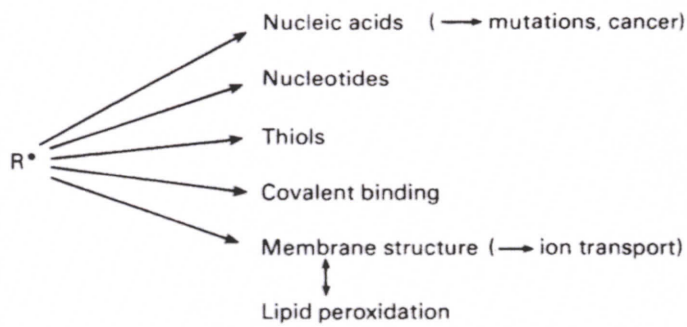

Figure 11: Free radicals and cellular injury (Slater, 1984)

Legend:
Free radicals and cellular injury Major routes are shown in which a reactive free radical (R') can interact with neighboring components in cells to disturb their metabolic function (s) are indicated by the arrows (Slater, 1984)

Oxidative damage to DNA, proteins, and other macromolecules accumulates with age and has been postulated to be a major, but not the only, type of endogenous damage leading to aging, superoxide and hydroxyl radical, which are mutagens produced by radiation, are also by products of normal metabolism. Lipid epoxides, lipid hydroperoxides, lipid alkoxyl and peroxyal radicals, and enals. Transfer of energy from lights, the respiratory burst from neutrophils, or lipid perodication can produce singlet oxygen, a high –energy and mutagenic form of oxygen. Four endogenous sources appear to accounts for most of the oxidants produced by cells, (i) as a consequence of normal aerobic respiration , mitochondria consume O2, reducing it by sequential steps to produce H2O, (ii) phagocytic cells destroy bacteria or virus infected cells with an oxidative burst of nitric oxide (NO). Chronic infection by viruses, bacteria or parasites results in chronic inflammation, which major risk factor for cancer, (iii) Peroxisomes, which are organelles responsible for degrading fatty acids and other molecules, produce H2O2 as a by-product, which is then degraded by catalase. (iv) Cytochrome P450 enzymes in animals constitute on of the primary defense systems against natural toxic chemicals from plants the major source of dietary toxins (Ames, et al., 1993).

(Caporaso, 2003), the action of modulators of oxidative DNA damage is the focuses of intense study and contraversory, Lung cancer is a logical disease for evaluating oxidative damage and the role of free radicals because the etiologic agents for long cancer are tobacco carcinogens that are known to damage the DNA.

Oxygen radicals in relations to Brain

Oxygen radical production may be especially harmful to the injured brain because levels of endogenous antioxidant enzymes [including superoxide dismutase (SOD), catalase, glutathione], and antioxidant vitamins (e.g., alpha-tocopherol, and ascorbic acid) are normally not high enough to match excess radical formation, after ischemia reperfusion, enhanced production of ROS overwhelms endogenous scavenging mechanisms and directly damages lipids, proteins, nucleic acids, and carbohydrates(Aneesh B. Singhal, 2006).

Inflammatory mediators such as cytokines and associated ROS are responsible for the pathologic changes related to TSE (Transmissible spongiform encephalopathy's) (Drisko, 2002).

Smoking, Alcohol and their effect on production of free radicals

Cigarette smoking is the major risk factor for several forms of lung disease, including chronic obstructive pulmonary disease (COPD) Cigarette smoking, which exposes the lung to high concentrations of reactive oxidant species (ROS). Recent studies indicate that ROS interfere with protein folding in the endoplasmic reticulum and elicit a compensatory response termed the ''unfolded protein response'' (UPR). The importance of the UPR lies in its ability to alter expression of a variety of genes involved in antioxidant defense, inflammation, energy metabolism, protein synthesis, apoptosis, and cell cycle regulation. The gaseous and particulate phases of cigarette smoke contain more than 4,500 separate compounds, many of which are highly toxic reactive oxygen/nitrogen species (RONS) and xenobiotic materials (Kelsen, et al., 2008).

It is estimated that only a minority (i.e., 15–35%) of chronic, continuous cigarette smokers develop COPD (14, 15). That the majority of long-term smokers do not develop COPD suggests that failure of compensatory mechanisms that protect the lung from ROS or xenobiotic materials contributes to development of the disease (Kelsen, et al., 2008)

Recent studies indicate that a complex molecular cascade termed the ''unfolded protein response'' (UPR) plays an important role in the regulation of expression of a variety of antioxidant, xenobiotic metabolizing and pro- and anti-inflammatory genes (Kelsen, et al., 2008).

There is little doubt that excessive consumption of alcohol over a considerable period of time may lead to an impairment of cognitive function, specific alcohol-related disorders such as Wernicke-Korsakoff syndrome (WKS), hepatic encephalopathy, and pellagra cause clinical dementia syndromes, but when these have been excluded there are still a number of alcoholics who have cognitive deficits (Harper, 2009)

[Shi and Collaegues (W. Liu, et al., 2008; H. Shi & Liu, 2007; X. Y. Shi, Tang, Sun, & He, 2006)] Production of acetaldehyde during alcohol metabolism, which through its interactions with proteins and lipids also can lead to radical formation and cell damage. Damage to the mitochondria resulting in decreased ATP production, effects on cell structure (e.g., the membranes) and function caused by alcohol's interactions with either membrane components (i.e., phosphate containing lipids [phospholipids]) or enzymes and other protein components of the cells.

Alcohol-induced oxygen deficiency (i.e., hypoxia) in tissues, especially in certain areas of the liver lobules (i.e., the pericentral region), where extra oxygen is required to metabolize the alcohol.

For more information on alcohol-induced hypoxia in the liver and its consequences (Wu & Cederbaum, 2003):

Alcohol's effects on the immune system which lead to altered production of certain signaling molecules called cytokines, which in turn lead to the

activation of an array of biochemical processes. production and its consequences, alcohol-induced increase in the ability of the bacterial molecule endotoxin to enter the bloodstream and liver, where it can activate certain immune cells.

Alcohol-induced increases in the activity of the enzyme cytochrome P450 2E1 (CYP2E1), which as described in the section "Systems Producing ROS" metabolizes alcohol and other molecules and generates ROS in the process (Wu & Cederbaum, 2003).

Alcohol-induced increases in the levels of free iron in the cell (i.e: iron that is not bound to various proteins), which can promote ROS generation, as described in the section "Role of Metals biochemical reactions generating an alcohol-derived radical (i.e., the 1-hydroxyethyl radical) Conversion of the enzyme xanthine dehydrogenase into a form called xanthine oxidase, which can generate ROS(Wu & Cederbaum, 2003)

Many of these processes operate concurrently, and it is likely that several, indeed many, systems contribute to the ability of alcohol to induce a state of oxidative stress (Wu & Cederbaum, 2003).

Based on the wealth of evidence that has accumulated over the past decade, it is clear that free radicals, and the resulting oxidative stress, are involved in cell death and brain injury after stroke (H. Shi & Liu, 2007)

ANTIOXIDANTS

Antioxidant supplements and diets have long been advocated for the treatment of rheumatoid arthritis (RA), osteoarthritis (OA) and other inflammatory arthritis. However, the value of antioxidants in the prevention and treatment of a wide range of serious diseases including stroke, cancer, diabetes, cataracts, Parkinson's disease, Alzheimer's disease and arthritis has been questioned in the light of more recent negative research findings and studies suggesting that antioxidant properties may be absent or reduced in vivo, may only be important in those with a deficiency or may even be harmful. (Canter, Wider, & Ernst, 2007)

Antioxidant is 'any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate'. This definition includes compounds of a non-enzymatic as well as an enzymatic nature. Clearly, the diversity of antioxidants matches that of pro-oxidants (Sies, 1997).

A first line of defense against reactive oxygen species is, of course, protection against their formation, i.e. prevention. There are numerous strategies in biology designed to evade oxidative stress, ranging from the plankton that descends from the surface of the seawater to lower levels of solar irradiation, to the packaging of DNA in chromatin to shield the genetic material by providing alternative targets. Microbes have developed specialized strategies to prevent oxygen- dependent killing by phagocytes. There are many enzymatic systems in cells and body fluids to control the level of reactive species which otherwise might generate a cascade of products which, in turn, would lead to attacking oxidants (Sies, 1997).

Nitric oxide

The role of nitrous oxide in regulating vascular tone, superoxide in fibroblast proliferation and hydrogen peroxide in the transcription of cytokines IL-2 and TNF-alpha. During inflammation, oxidation modifies low-density lipoproteins, inactivates Alpha-1-protease inhibitor, damages DNA and causes lipid peroxidation. Reactive oxygen species also damage cartilage and the extracellular matrix and inhibit collagen and proteoglycan synthesis. (Canter, et al., 2007).

(Moncada & Higgs, 2006),by 1987 it had become apparent that the generation of NO was not only taking place in the vascular wall, but was probably a widespread mechanism with far-reaching biological significance. NO pathway in the central nervous system, where we then identified the NO syntheses and determined its dependency on

calcium, In the late 1980s, NO was identified as the inhibitory mediator of nonadrenergic, noncholinergic (NANC) neurotransmission

in peripheral nerves. This 'nitrergic' neurotransmission has been demonstrated in the gastrointestinal tract where it is responsible for NANC-mediated relaxations of the gastric fundus, in the corpus cavernosum where it brings about relaxation of this tissue, resulting in penile erection, and in the trachea and the bladder where it contributes to NANC induced relaxation, nitric oxide (NO), a multifunctional small radical molecule, is involving in a number of regulatory mechanisms, including vasodilatation, neurotransmession and nueromodulation, inhibition of platelet aggregation and modulation of leukocyte adhesion, on the basis of the growing evidence for the biological importance of NO and the L-arginine-NO pathway in neural and vasoregulatory mechanisms of the brain. (N. Bitterman & Bitterman, 1998)

Dietary Antioxidants.

The effect of dietary intake of the antioxidants ascorbate,tocopherol, and carotenoids is difficult to disentangle by epidemiological studies from other important vitamins and ingredients in fruits and vegetables. Nevertheless, several arguments suggest that the antioxidant content of fruits and vegetables is a major contributor to their protective effect. (i) Biochemical data, discussed above, show that oxidative damage is massive and is likely to be the major endogenous damage to DNA, proteins, and lipids. (ii) Oxidative damage to sperm DNA is increased when dietary ascorbate is insufficient. (iii) Epidemiological studies and intervention trials on prevention of cancer and cardiovascular disease in people taking antioxidant supplements are suggestive, though larger studies need to be done. Small-molecule dietary antioxidants such as vitamin C (ascorbate), vitamin E (tocopherol), and carotenoids have generated particular interest as anticarcinogens and as defenses against degenerative diseases. Most carotenoids have antioxidant activity, particularly against singlet oxygen, and many, including Beta/-carotene, can be metabolized to vitamin A (retinal). Earlier papers have called attention to a number of previously neglected physiological antioxidants, including urate, bilirubin, carnosine,

and ubiquinol. Ubiquinone (CoQ1o), for example, is the critical small molecule for transporting electrons in mitochondria for the generation of energy. Its reduced form, ubiquinol, is an effective antioxidant in membranes. Optimal levels of dietary ubiquinone/ubiquinol could be of importance in many of the degenerative diseases.(Ames, et al., 1993)

3.2.3 Basic Aspects Of Stroke

STROKE

Definition of stroke

Stroke was defined according to the World Health Organization (WHO), criteria as rapidly developed clinical signs of focal disturbance of cerebral function, lasting for 24 h, or leading to death, with no other apparent cause than cerebrovascular disease. (Abo K, 1980)

The term stroke denotes a sudden event in which a disturbance of CNS function occurs due to vascular disease, the annual incidence of stroke is 2 per 1000 of the general population in UK, but is much commoner in the elderly. These events can be classified clinically into *completed strokes, evolving storks, or transient ischemic attacks* in which the CNS disturbance lasts for less than 24 hours. Transient ischemic attack is a major risk factor for cerebral infarction; most attacks are due to circulatory changes in the CNS occurring as the result of disease in the heart or extracranial arteries.

The clinical features of stroke depend on the localization and nature of the lesion. (J.C.E.UNDERWOOD, 1992)

The consequence of stroke can be understood in the context of the international classification model; in this model health condition represents both healthy body systems and disorders or disease. This concept includes the damage that occurs in the brain tissue as a result on an ischemic blockage or hemorrhagic stroke, as well as other co morbidities. This damage often affects performance at the level of body function and structures in the ICF model, including motor weakness in hemi paretic pattern, hyper tonicity, impaired motor control, sensory loss, decreased cognition, and the effects of de conditioning. (Kluding & Gajewski, 2009).

Epidemiology of stroke and TIA:

Stoke is the second most common cause of death worldwide and major cause of long-term disability, with a great impact on public health. Increasing age, hypertension, cigarette smoking, alcohol abuse, diabetes mellitus, hypercholesterolemia, and history of coronary heart disease, which contributes in to the risk of stork. M&M, particularly in older people, with an estimated incidence rate of 3-5 per 1000 in those aged 45-84 years (McManus, Craig, McAlpine, Langhorne, & Ellis, 2009) (Mitsios, et al., 2006) .

According to the European Scientific Community About one million strokes occur each year in the European Union. Indeed, about 25% of men and 20% of women can expect to suffer a stroke if they live to be 85 years old. As a cause of death worldwide, stroke is second only to coronary heart disease. Although stroke is a major cause of death, mortality data underestimate its true burden. This is chronic disability. Since stroke causes disability more often than death, stroke patients frequently require long hospital stays followed by ongoing support in the community, or nursing home care. Stroke is consequently a major drain on health care funding. Stroke is the number one cause of disability in the European Union.(Meairs, et al., 2006).

Traumatic brain injury (TBI) is one of the most disabling injuries in the population, with 1.5 million American's new case each year and 5.3 million Americans overall requiring long term daily care as a result of their injuries (Spaethling, Klein, Singh, & Meaney, 2008). Early impairment of cerebral blood flow in patients with severe head injury correlates with poor brain tissue O delivery and may be an important cause of ischemic brain damage (Menzel, et al., 1999)

Strokes Categories

Stroke can be categories into 4 basic stroke types (D.Hill, 2008):

Ischemia *(Ischemic stroke and transient ischemic attack 85% of all strokes).*

Intercerebral hemorrhage *(7%-8% of all strokes)*

Subarchnoid stroke *(7%-8% of all strokes).*

Cerebral venous sinus thrombosis *(<<1% of all strokes).*

In turn of each these stroke have common risk factors which are *(DM, Hypertension, smoking)*Which in generally they underline many storks.

The Trail of ORG 10172 in Acute Stroke Treatment *(TOAST),* classification system is typical used in categorized ischemic stroke into larger artery disease cardio embolic stroke, Lacunar disease, and others.

Risk factors: (Jain, 2004)

Various risk factors for stroke are:

- Aging
- Alcohol
- Atherosclerosis involving major vessels
  Atherosclerosis involving aortic arch
- Carotid stenosis
- Cardiovascular disease
  Heart disease, arterial fibrillation, endocardities, left ventricular hypertrophy, mitral valve prolapse, myocardial infarction, patent foramen ovale.
- Coagulation disorders
- Cold
- Endocrine disorders
- Diabetes mellitus
- Hypothyroidism
- Female sex
- Genetic
- Angiotensin-converting enzyme gene deletion polymorphism

- Genetically determined cardiovascular, hematological and metabolic disorders causing stroke
- Hemorrheological disturbances
- Increased blood viscosity, elevated hematocrit, red blood cells disorders, and leukocytosis.
- Heredity; parental history of stroke is associated with increased stroke risk in the offspring.
- Hyperlipidemia
- Hypertension
- Hypotension
- Lack of physical activity
- Metabolic disorders; hyperuricemia, hyperhomocysteinemia
- Migraine
- Nutritional disorders; high salt intake, malnutrition, vitamins deficiency
- Obesity
- Psychological factors; anger, aggression, stress.
- Pregnancy
- Race: strokes more common in black American than whites.
- Raised serum fibrinogen levels
- Sex: strokes more common in male than female
- Sleep related disorders: sleep apnea and snoring.
- Smoking
- Transient ischemic attacks.

The interaction between risk factors and causes of stroke:(Jain, 2004)

Table 4: Interaction between risk factors and stork (Jain, 2004)

Stages in the Evolution of a Cerebral Ischemic Episode	
I *Transient Ischemic Attacks (TIA)*	Minutes to 24h
II *Reversible Ischemic neurological deficit (RIND)*	Hours
III *Prolonged reversible ischemic neurological deficit (PRIND) (Stroke in evolution)*	Days
IV *brain Infarction with fixed Neurological deficit (complete stroke)*	Hours to Months
V *Chronic post stroke*	More than one year after onset

There are 2 forms of intrinsic cerebral hemorrhage, primary **ICH** *internal cerebral hemorrhage*, which has a predilection to affect the striatum, thalamus, midbrain, pons, and cerebellum.

SAH Subarachnoid *hemorrhage*, which is referred to bleeding beneath the arachnoids covering of the brain surface and within the contained cisterns. The most common age is between the fifth and seventh decades of life.(Frank H. Netter, 2005)

Spontaneous intracerebral hemorrhage

Spontaneous Intercerebral hemorrhage which accounts for 15-20% of all strokes, has a poor prognosis, paradoxically, hemorrhaged blood is highly toxic to the brain, most likely because of exposure to free heme (Iron protoporphyrin IX, a pro-oxidant) liberated from hemoglobin and other hemoproteins. Because blood is released into extracellular spaces, and heme cannot be recycled, heme metabolism is critical for the resolution of ICH. (Wang & Dore, 2007)

Several risk factors play the role in eithopathogenesis of spontaneous intracerebral hemorrhage (ISCH), the role of DM, is controversial and contradictory in this field, according to the study which was done in the Czech Republic, (Olomouc region), the aim was to be able to assess the role of DM present prior to a hemorrhagic stork in the patients with (SICH), and to compare it with prevalence in SICH patients to the finding in general population (as represented by the control group subjects with low back pain), according to some authors, DM does not rank among its risk factors. Moreover, a reduced ICH prevalence in diabetic patients was reported in some papers, they found out 1.5-up to 6-fold lower ICH occurrence in diabetic, when compared with non-diabetics.

On the other hand, an opposite opinion, considering DM to be a proven as a risk factor for SICH, at least in young patients aged (18-49 years), can be also encountered.

Based on the findings that the researchers in Olomouc they couldn't confirm the protective effect of DM leading to the SICH prevalence decrease. This was explained as a possible effect of the specific angiopathy induced by DM and characterized by a thickening of the basement membrane of small cerebral vessels and proliferation of the endothelium, as well as by the increase of platelet aggregability, coagulability and plasmoinogen activator inhibitors levels, and decrease of the fibrinolytic activity in DM patients. And the studied concluded that the spinal problem do not interfere with vascular risk factors. (R.Herzig.I, 2007)

Intracerebral Hemorrhage

Intracerebral hemorrhage is focal bleeding from a blood vessel in the brain parenchyma. The cause is usually hypertension. Typical symptoms include focal neurologic deficits, often with abrupt onset of headache, nausea, and impairment of consciousness. Diagnosis is by CT or MRI. Treatment includes BP control, supportive measures, and, for some patients, surgical evacuation (Jones, 2005).

Most intracerebral hemorrhages occur in the basal ganglia, cerebral lobes, cerebellum, or pons. Intracerebral hemorrhage may also occur in other parts of the brain stem or in the midbrain.(Frank H. Netter, 2005)

Intracerebral hemorrhage usually results from rupture of an arteriosclerotic small artery that has been weakened, primarily by chronic arterial hypertension. Such hemorrhages are usually large, single, and catastrophic. Use of cocaine or, occasionally, other sympathomimetic drugs can cause transient severe hypertension leading to hemorrhage. Less often, intracerebral hemorrhage results from congenital aneurysm, arteriovenous or other vascular malformation: Vascular Lesions in the brain, trauma, mycotic aneurysm, brain infarct (hemorrhagic infarction), primary or metastatic brain tumor, excessive anticoagulation, blood dyscrasia, or a bleeding or vasculitic disorder.(Frank H. Netter, 2005).

ICH causes infiltration of the brain by blood components that induce an inflammatory response. (Wang & Dore, 2007)

Subarachnoid Hemorrhage (SAH)

Subarachnoid hemorrhage is sudden bleeding into the subarachnoid space. The most common cause of spontaneous bleeding is a ruptured aneurysm. Symptoms include sudden, severe headache, usually with loss or impairment of consciousness. Secondary vasospasm (causing focal brain ischemia), meningismus, and hydrocephalus (causing persistent headache and obtundation) are common. Diagnosis is by CT or MRI; if neuroimaging is normal, diagnosis is by CSF analysis. Treatment is with supportive measures and neurosurgery or endovascular measures, preferably in a referral center.(van Gijn & Rinkel, 2001)

Subarachnoid Hemorrhage (SAH), mostly form aneurysms, accounts for only 3% of all strokes, but for 5% of stroke deaths and for more than one-quarter of potential life years lost through stroke.(van Gijn & Rinkel, 2001).

The incidence of SAH has remained stable over the last 30 years, in meta-analysis of relevant studies, the pooledincidence rate was 10.5 per 100 000 person years. There seemed to be a decline over time, but this was caused by diagnostic bias. That more recent studies reported lower incidence rates than older studies in which CT is applied to all patients, the incidence is calculated to be 5.6 per 100 000 patients a year. Gender race and region have a marked influence on the incidence of SAH, woman have a 1.6 times [95%confidence interval (CI)] higher risk than men, and black people a 2.1 times (95% CI 1.3-3.6) higher than white men. In Finland and Japan, the incidence rates are much higher than in other parts of the world. (van Gijn & Rinkel, 2001)

Neurons

Neurons or nerve cells vary, considerably in size and appearance within the CNS. All possess a cell body, and axon and dendrites. The cell body or perikaryon is easily seen by light microscopy. The perikaryon contains neurofilaments, microtubules, lysosomes, mitochondria, complex stacks of rough endoplasmic retinaculum, free ribosome's and a single nucleus with a prominent nucleolus, some group of neurons contain the pigment neurolmelanin and are readily identifiable with the naked eye as darkly colored nuclei such as the one seen in susbstantia niagra. Axon and dendrite are the neuronal processes, which convey electrical impulses from and towards the perikaryon respectively. These processes vary enormously in size and complexity and maybe difficult to identify. Neurons: can undergo various reactive changes to cell injury(J.C.E.UNDERWOOD, (1992)).

Injury to the adult central nervous system (CNS) is devastating because of the inability of central neurons to regenerate correct axonal and dendritic connections. The consequences of injury are not just breaks in communication between healthy neurons, but a cascade of events that can lead to neuronal degeneration and cell death(Horner & Gage, 2000).

As reported by RamoÂn y Cajal1 in 1928, Tello showed in 1911 that adult CNS neurons could regrow if they were provided access to the permissive environment of a conditioned sciatic nerve. Seventy years passed before Aguayo and colleagues replicated these studies with new methods that definitively confirmed that adult CNS neurons have regenerative capabilities. This finding revealed that the failure of CNS neurons to regenerate was not an intrinsic deficit of the neuron, but rather a characteristic feature of the damaged environment that either did not support or prevented regeneration.(Horner & Gage, 2000)

HEMOGLOBIN

The heme proteins myoglobin and hemoglobin maintain a supply of oxygen essential for oxidative metabolism. myoglobin, a monomeric protein of red muscle, Stores oxygen as a reserve against oxygen deprivation. Hemoglobin, a tetrameric protein of erythrocytes, Transports O2 to the tissues and returns CO2 and protons to the lungs. Cyanide and carbon monoxide kill because they disrupt the physiologic function of the heme proteins cytochrome oxidase and hemoglobin, respectively. The secondary-tertiary structure of the subunits of hemoglobin resembles myoglobin. However, the tetrameric structure of hemoglobin permits cooperative interactions that are central to its function. (Murray, 2000).

Hemoglobin actively regulates oxygen transport through the oxygen-hemoglobin (oxyhemoglobin) dissociation curve which describes the relation between oxygen saturation or content of hemoglobin and oxygen tension at equilibrium, Haldane effect, results from the fact that the combination of oxygen with hemoglobin causes it to become a stronger acid, this displaces CO2 from the blood by 2 ways: when there is more acid, hemoglobin has less of a tendency to combine with CO2 to form carbhemoglobin. Increased acidity of the hemoglobin causes it to release an excess H+ ions and these, in turn bin with bicarbonate ion to form carbonic acid, which then dissociates into water and CO2, which released from the blood into the alveoli. (Jain, 2004)

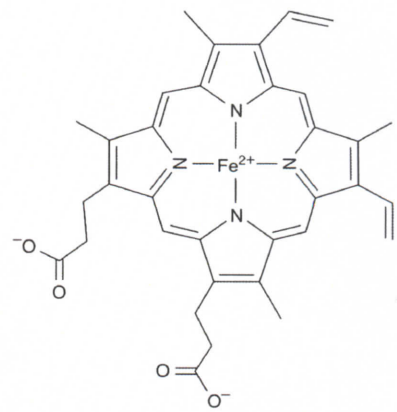

Figure 12: Heme (Murray, 2000)

Legend:
Heme. The pyrrole rings and methylene bridge carbons are coplanar, and the iron atom (Fe2+) resides in almost the same plane. The fifth and sixth coordination positions of Fe2+are directed perpendicular to—and directly above and below—the plane of the heme ring. Observe the nature of the substituent groups on the βcarbons of the pyrrole rings, the central iron atom, and the location of the polar side of the heme ring (at about 7 o'clock) that faces the surface of the myoglobin molecule. (Murray, 2000)

BBB

The BBB; (Blood brain barrier), depends on the unique characteristics of the brain capillaries. Continuous tight junctions join the endothelial cells of brain capillaries. In addition, most brain capillaries are completely surrounded by a basement membrane and by the processes of supporting cells of the brain, called astrocytes.

The *BBB* permits passage of essential substances while excluding unwanted materials. Reverse transport systems remove materials from the brain. Large molecules such as proteins and peptides are largely excluded from crossing the blood-brain barrier. Acute cerebral lesions, such as trauma and infection, increase the permeability of the blood-brain barrier and alter brain concentrations of proteins, water, and electrolytes. Due to its unique structure, the blood–brain barrier (BBB) is capable of limiting the penetration of a variety of substances from the blood into the brain. The BBB plays an important role in the homeostasis and is generally seen as a defense mechanism that protects the brain against various molecules that may enter the BBB. The BBB is composed of endothelial cells, which form a diffusion barrier, due to the presence of tight junctions that firmly connect endothelial cells. Due to the development of small molecular weight tracers that enter the damaged BBB, disruption was found to be associated with various neurological disorders such as migraine, post concussion syndrome, multiple sclerosis and epilepsy. BBB disruption has been shown both in human as well as in animal studies (van Vliet, et al., 2007)

Moreover, Friedman and colleagues showed that focal opening of the BBB

by direct cortical application of albumin-containing solution can lead to the generation of an epileptic focus in rats

Leakage of the blood–brain barrier (BBB) is associated with various neurological disorders, including temporal lobe epilepsy (TLE). However, it is not known whether alterations of the BBB occur during

epileptogenesis and whether this can affect progression of epilepsy.(van Vliet, et al., 2007)

Cerebrospinal Fluid–Brain Barrier *(CSF)*

The ependymal cells covering the choroid plexus are linked together by tight junctions, forming a blood-CSF barrier to diffusion of many molecules from the blood plasma of choroid plexus capillaries to the CSF. Water is transported through the choroid epithelial cells by osmosis. Oxygen and carbon dioxide move into the CSF by diffusion, resulting in partial pressures roughly equal to those of plasma. The high sodium and low potassium contents of the CSF are actively regulated and kept relatively constant. (Jain, 2004)

The consequences of cerebral ischemia on the structure and function of the brain depend largely on the degree and duration of reduced CBF. In rodent models, a 20-30% decrease in CBF results in decreased protein synthesis. A 50% decrease in CBF results in increased lactate production and concomitant glutamate increase. When CBF reaches 20% of its normal rate, brain cells begin to lose their ionic gradients and undergo depolarization, which coincides with irreversible neuronal damage (H. Shi & Liu, 2007)

CARDIOGENIC FACTORS

Several type of cardiac disease leads to cerebral embolism; cardiac arrhythmias, ischemic heart disease, valvular disease, dilated cardiomyopathies, arterial septal abnormalities and intracardic tumors.

Cardiac arrhythmias include chronic and paroxysmal arterial fibrillation (AF) and sick sinus syndrome *(in particular Brady tachycardia syndrome).* Are the most embologenic rhythms. Often, stroke is first sign of (AF). Because this arrhythmia is often intermittent.

Patent foramen ovale (PFO) and atrial septal aneurysm are risk factor for stroke.(Frank H. Netter, 2005)

Table 5: A meta-analysis of Patent foramen ovale (Jones, 2005)

A meta-analysis of case control studies comparing younger patient (younger than 55 years) o ischemic stroke and non-stroke control patients showed an odds ration for stroke of 3.1 for PFO *(Patent foramen ovale)* And have 6.1 for atrial septal aneurysm. Potential mechanisms for stroke included paradoxical embolism, direct embolization from thrombi formed within the *(PFO)* or atrial septal aneurysm, and thrombus formation caused by atrial rhythmias thought to be more prevalent in this population. (Jones, 2005)

Atrial myxomas, although rare, are important cause of embolic strokes. These tumor emboli frequently affect the vasa vasorum lading to the development of multiple and peripheral cerebral aneurysms similar to mycotic aneurysms. (Frank H. Netter, 2005)

Large artery occlusive disease

Atherosclerosis causes stenosis or occlusion of the extracranial and intracranial arteries and is directly responsible for a significant percentage of cerebral ischemic events. Atheroma formation involves the progressive deposition of fatty materials and fibrous tissue in the subintimal layer of the large and medium arteries, occurring most frequently at branching points.

Intraplaque hemorrhage, subintimal necrosis with ulcer formation, and calcium deposition can cause enlargement of the atherosclerotic plaque with consequent worsening of the degree of arterial narrowing. This disruption of the endothelial surface triggers thrombus formation within the arterial lumen, due to the activation of nearby platelets by the sub endothelial collagen. (Frank H. Netter, 2005)

(Lacunes) Stroke

Lipohyalinosis and fibrinoid necrosis affecting the small arteries are the anatomic substrate of small vessels disease. Occlusion of these arteries

causes small *(1-20 mm)*, discrete, and often-irregular lesions called lacunes to develop. Lacunes occur most often in the basal ganglia, thalamus, pons, internal capsule, and cerebral white matter. Arterial hypertension and diabetes are the main risk factor. lacunar stroke maybe embolic (5-7), cardiombolic stroke may include mural thrombus after myocardial infarction, atrial fibrillation, valvular heart disease, and aortic arch ateroembolism. (Frank H. Netter, 2005)

THE ABCD AND ABCD2 SCORES IN TIA PATIENTS

Several validate risk scores exist for long-term risk of stroke following TIA, the medium risk and more, recently, the short-term risk. The ABCD score (age, blood pressure, clinical features, duration) was derived to predict the risk of stroke at 7 days and was validated in several independent cohorts including a population-based cohort of patient with TIA in Oxford Vascular Study (OXVASC). And the ABCD2 score refined the ABCD score, by adding a point to DM, in order to best predict the risk of stroke in the first 48h. (Koton & Rothwell, 2007)

According to the studies which were done by (Koton & Rothwell, 2007), a which they studied the association between ABCD and ABCD2 scores, the presence of □ 50% ipsilateral carotid stenosis, or AF (Arterial fibrillation), and risk of stroke at 7 days. Their result were, done and among 285 TIA patients (from 559 referrals of possible TIA), 69 (24%) had either □ 50% carotid stenosis (n=29) or AF (n=42), or both (n=2).

ENDOTHELIAL AND ITS LINK TO PATHOGENETIC OF ATHEROSCLEROSIS

The endothelium is a crucial vascular structure not only because it serves as a barrier between flowing blood and vascular wall but also because it produces mediators regulating vascular growth platelet function and coagulation. In addition, it alerts vasomotor tone by synthesizing and metabolizing vasoactive substances including an endothelium- derived hyperpolarizing factor, prostacyclin, and most notably, endothelium-

derived relaxing factor (EDRF), which has been identified as a nitric oxide (NO) or a related compound. There is nothing new in the notion that endothelium is central to the pathogenesis of atherosclerosis; how ever the old assumption that endothelial desquamation precedes lesion development has given way to the concept of dysfunction of the endothelium since even advanced lesions may be covered by intact, albeit morphologically alerted, endothelial cell layers. (Zeiher, 1996)

In general recognized that endothelium produces a number of substances that can modulate local platelet adhesion and aggregation antiplatelet pavacine substances includes two compound's that are also vasodilators. These prostacyclin (PGI2) and endothelium derived relaxing factor nitric oxide (EDRF/NO). Tissue beneath the endothelium "Subendothelium" contains collagen fibrils. In early 1960s, it was established that such collagen could cause platelets adhesion and aggregation in vivo.

It is now well known that shear can activate platelets perhaps by up regulation important proaggregant molecules on the surface of platelets.

There is a potential clinical importance of modest, non denuding endothelial damage in encouraging local adhesion/aggregation, specially where passing platelets may already have been activated by up streams events, such events occur in coronary disease and in diabetes, activated or hyperaggregable platelets are also found between transient ischemic attacks and after complete ischemic stroke.(Rosenblum, 1997)

Endothelial cells produce NO by activation of endothelial nitric oxide eNOS. Nitric oxide is a double-edge sword in cerebral ischemia and reperfusion: it can be both protective and deleterious, depending on where and when it is generated. Immediately after brain ischemia, NO release from eNOS is protective mainly by promoting vasodilatations.(H. Shi & Liu, 2007)

Atherosclerosis is the leading cause of death in Western society, several lines of evidence suggest that oxidized lipoprotein play an important role in the etiology of this disease, Oxidized LDL *(low-density lipoprotein),* is a powerful atherogenic agent that is able to bypass the normal feedback-regulated cellular cholesterol uptake by peripheral cells and lead to foam cell production, Oxidized LDLs potentate monocyte-endothelial cell interaction, regulate cytokine expression and stimulate chemotactic factor expression, they have also been shown to induce apoptosis in vascular cells. (Kudchodkar, et al., 2000)

The development of atherosclerosis is strongly associated with dysplidaemia is related to aortic atherosclerosis detected by transoesophageal echocardiography which have been regarded as important predictors of ischemic stroke, although these atherosclerotic plaques have been reported to be sources of cerebral emboli, particularly when they are ≥ 4mm in thickness, the relation of atherosclerosis plaques to different subtypes of ischemic stroke remains undetermined (Matsumura, et al., 2002)

Atherosclerosis is being viewed as an inflammatory disease, the metabolic stress imposed on the endothelium itself seem to recruit inflammatory cells into the atherosclerotic vascular wall via stimulation of redox sensitive genes. (Zeiher, 1996)

The arterial wall is composed of a highly ordered structure of cells and extracellular matrix (ECM). In the intimal layer, endothelial cells sit upon a basement membrane rich in laminin, fibronectin and type IV collagen. In the media, individual smooth muscle cells (SMCs) are surrounded by a basement membrane, and are in turn embedded in fibrillar types I, III and V collagen, type XVIII collagen,

Fibronectin and proteoglycans. In elastic arteries, each layer of SMCs is separated by a well-defined, fenestrated elastic lamina. The outermost layer, the adventitia, is rich in fibroblasts, collagen types I and III and elastin. After arterial injury and during atherosclerotic plaque development,

there are pronounced changes in the composition of the ECM. A collagen molecule in the initiation and progression of atherosclerosis is expanding at an increasing rate. Once thought to be biologically inert, collagens are now known to be bioactive components of the ECM, which interact with and exert profound effects on many cell types within the vessel wall. Moreover, it has become evident that these effects are heavily dependent on the geometric organization of collagen molecules within the ECM(Zeiher, 1996)

Yet despite their strength, collagens are highly vulnerable to proteolytic digestion, remodeling and mineralization, complications which mark advances in the severity of atherosclerosis and increases in the risk of detrimental clinical events.(Eser Adiguzel, 2009)

On the basis of epidemiologic data, it has been predicted that each 1 percent reduction in the level of low-density lipoprotein (LDL) cholesterol results in a reduction of 1.0 to 1.5 percent in the risk of major cardiovascular events. In trials of LDL-lowering strategies, a reduction of 12 to 38 percent in the LDL level has resulted in a relative reduction in risk of 19 to 35 percent (Matsumura, et al., 2002)

ATRIAL FIBRILLATION

Atrial fibrillation is the most common arrhythmia encountered in clinical practice, accounting for approximately one third of all hospitalizations for cardiac rhythm disturbances. It has been estimated that 2.2 million Americans have paroxysmal or persistent AF, but the actual number may be higher. The prevalence of AF increases with age, reaching as high as 9% in octogenarians. During the past 20 years, there has been a 66% increase in hospital admissions for AF due to a combination of factors, including the aging of the population, a rising prevalence of chronic heart disease, and

more frequent diagnosis through use of ambulatory monitoring devices. Atrial fibrillation is associated with an increased risk of stroke, heart failure, and all-cause mortality, especially in women. The mortality rate of patients with AF is higher than that of patients in normal sinus rhythm and is linked to severity of underlying heart disease. The guidelines for management of patients with AF recommend as a Class I indication that antithrombotic therapy for patients with atrial flutter follows the same approach as for patients with AF, given the evidence of their comparable risk of thromboembolism. Accordingly, these performance measures also apply to patients with atrial flutter who do not have valvular heart disease.

Given the morbidity, mortality, and costs associated with AF and atrial flutter, the ACC *(American college of cardiology)*, AHA *(American heart association)*, and Physician Consortium chose this topic for performance measures both to raise the level of awareness of current guidelines and to provide tools physicians can use in practice to improve the quality of care provided to patients with non-valvular AF and atrial flutter.(Estes, et al., 2008)

HYPERTENSION AND DM

Cardiovascular diseases (CVDs) are the major causes of mortality in persons with diabetes. Hypertension is approximately twice as frequent in patients with diabetes compared with patients without the disease. Conversely, recent data suggest that hypertensive persons are more predisposed to the development of diabetes than are normotensive persons. Furthermore, up to 75% of CVD in diabetes may be attributable to hypertension, leading to recommendations for more aggressive treatment (i.e., reducing blood pressure to <130/85 mm Hg) in persons with coexistent diabetes and hypertension. Other important risk factors for CVD in these patients include the following: obesity, atherosclerosis, dyslipidemia, microalbuminuria, endothelial dysfunction, platelet

hyperaggregability, coagulation abnormalities, and "diabetic cardiomyopathy." The cardiomyopathy associated with diabetes is a unique myopathic state that appears to be independent of macrovascular/microvascular disease and contributes significantly to CVD morbidity and mortality in diabetic patients, especially those with coexistent hypertension. This update reviews the current knowledge regarding these risk factors and their treatment, with special emphasis on the cardio metabolic syndrome, hypertension, microalbuminuria, and diabetic cardiomyopathy. This update also examines the role of the renin-angiotensin system in the increased risk for CVD in diabetic patients and the impact of interrupting this system on the development of clinical diabetes as well as CVD.(Sowers, Epstein, & Frohlich, 2001)

Further more regarding the DM hypertension and Atherosclerosis since they come hand in hand. Studies of atherosclerotic coronary arteries from diabetic patients have uncovered increased expression of the mRNAs for types I and III collagen correlated with markers of cell proliferation including

Cdks and cyclins. These results are interesting because compared to non-diabetic patients with atherosclerosis, diabetics experience marked

enhancement of vascular collagen accumulation, modification of collagens by advanced glycation end products, and an increased incidence of restenosis. Thus, collagen remodeling may be linked to the increased severity of disease in diabetics.(Eser Adiguzel, 2009)

(RTPA, TPA, OR, PLAT)AND ASPIRIN AS FIRST INTAKE ON THE ONSET OF STROKE

More than ten years after its approval of (rtPA) recombinant tissue plasminogen activator, is still the only one approved therapy for acute ischemic stroke (AIS). Despite its potential, only 1-2% of all stroke patients receive rtPA, among the major problems are that relatively few candidate present within the window, and meet the clinical criteria. Stroke physicians

are frequently confronted with stroke patients who awaken with a deficit, or unable to provide the required information's due to aphasia or disorientation, at present, such patients are excluded from thrombolytic therapy, even if a CT-Scan is normal or has only minor ischemic changes. (Kohrmann, Juttler, Huttner, Nowe, & Schellinger, 2007).

There is a question that we might wonder since the trPA is applied to certain criteria of patients? Aspirin in a daily dose f 160 to 300 mg, initiated within 48 h of symptoms onset results in a net decrease in morbidity and mortality cause by acute ischemic stroke. (Strength of recommendation) SOR; A based on systemic review, regardless of availability of CT-Scan. (SOR: A based on meta-analysis). Aspirin is as effective as anticoagulants in this regard and causes less harm (SOR: A, based on a systemic review), but it should not be used in patients receiving thrombolytic therapy. Ibuprofen (Motrin) may decrease aspirin's effectiveness in acute ischemic stroke. (SOR: C, based on expert opinion) (Park, Smith, Wanserski, & Neher, 2009).

The Cochrane review confirmed that findings of a previous meta-analysis of the IST (International Stroke Trails) and CAST (Chinese Acute Stroke Trail). This meta-analysis included subgroup analyses, which found no difference in effect of treatment based on time from onset of symptoms (zero to 48 hours), age, sex, level of consciousness, history of AF, heparin administration, CT findings, or previous CT-Scanning. Patients with a hemorrhagic stroke who were inadvertently randomized did not appear to be harmed by aspirin (16% death or further stroke compared with 18% for the control group). A double-blind, randomized, placebo-controlled trail of 441 patients evaluated the effects of aspiring 325 mg daily for five days on progression of acute ischemic stroke compare with placebo. Aspirin didn't affect stroke progression during the treatment period, Its been recommended by the American Heart Association and the American Stroke Association Stroke Council recommended an initial aspirin dose of 325 mg within 24 to 48 hours of the onset of symptoms; however, not within 24 hours of thrombolytic therapy. The American College of Chest Physicians

recommended a recommended aspirin 160 mg to 325 mg daily started within 48 hours in patients not receiving thrombolytic therapy. (Park, et al., 2009)

MOLECULAR PATHOPHYSIOLOGY OF ACUTE ISCHEMIC STROKE

As we were mentioned earlier there is risks that contribute with stroke, the majority of strokes are cerebral ischemic infarction produced as a result of atherothrombosis or cardiac and artery- to artery embolism leading to sudden cessation of cerebral blood flow and oxygen-glucose deprivation in the corresponding vascular territory, and in every neurons die within the ischemic core tissue, while the majority of neurons survive for a slightly longer time in the surrounding penumbra. Cells at the infracted core are vulnerable and may die within minutes of ischemic onset; however, different brain areas have varying susceptibilities' to ischaemia and cell survival largely depends on the severity and duration of the ischemic incident.(Mitsios, et al., 2006), Acute ischemic stroke results in heterogeneous changes in cerebral blood flow and brain metabolism in the affected region. Ischemic penumbra is the initially viable tissue compromised by partially decreased cerebral blood flow and disturbed metabolism surrounding the severely damaged ischemic core (S. Liu, et al., 2004)

Cerebral ischemia causes heterogeneous changes in tissue oxygenation and cellular metabolism, with a region of decreased blood flow, the penumbra, surrounding a severely damaged ischemic core. Because oxygenation is central in ischemic neuronal death, it is critical to understand exactly what actual changes occur in interstitial oxygen tension (pO2) in ischemic regions during stroke, particularly the penumbra and ischemic core. Cerebral ischemia induces a complex series of molecular pathways involving signaling mechanisms, gene transcription, and protein formation. The final volume of the infracted tissue is determined by the surviving (H.

Shi & Liu, 2007). Penumbra, a region of potentially viable ischaemic tissue around the infracted core, where electrical activity is maintained and blood flow rate (more than 20–25% of regular blood flow) is sufficient for a certain degree of neuronal function and membrane integrity, with the degree of neuronal loss being proportional to the degree of cerebral blood flow reduction . The penumbra includes ischaemic areas that recover spontaneously and areas that progress to irreversible changes, unless effective treatment is used. After an ischaemic injury to the brain, the centre of the core is perfused at 10–12 ml/100 g/min or less while the ischaemic area around the centre (immediately surrounded by the penumbra) is critically hypoperfused at less than 18– 20 ml/100 g/min and is at risk of dying within hours, as the evolving infarction expands. The penumbra zone immediately surrounded by normal brain tissue is less likely to die, since it is perfused at a higher rate, although below the minimal normal rate of approximately 60-ml/100 g/min. There, selective gene expression and a decrease in protein synthesis are observed while in the area immediately surrounding the infracted core, perfused at just over 20 ml/100 g/min, lactic acidosis and cytotoxic edema take place.(Mitsios, et al., 2006)

The ischaemic core suffers an irreversible loss of ion homeostasis while in the penumbra the insult is either milder or of shorter duration. Neurons in the penumbra are mostly dysfunctional and unable to maintain full normal function but can be saved if reperfused in time.

In contrast, if blood flow drops below 10–15 ml/100 g/ min and does not return to normal, the infarct will expand and neuronal activity will cease. If the ischaemic state continues for an extended period, primary neuronal death occurs quickly in the core, with secondary death
developing gradually in the penumbra. (Mitsios, et al., 2006)

In cerebral ischaemia, the obstruction of cerebral blood flow results in a quick reduction of ATP availability, rapid failure of membrane ionic pumps and loss of ion homeostasis, release of glutamate, and neuronal calcium excess that activate a series of detrimental enzymatic cascades. All these

events occur within 1 or 2 h.(Mitsios, et al., 2006)

While irreversible cell death begins within minutes after stroke onset within regions of maximally reduced blood flow (the infarct "core"), for several hours there exists a surrounding "penumbra" of ischemic but non-infracted tissue that is potentially salvageable

The concept of an "ischemic penumbra" provides a rationale for the use of neuroprotective drugs and reperfusion techniques to improve outcome after acute ischemic stroke. Then again, the extent of penumbral tissue is thought to diminish rapidly with time, hence the therapeutic time window is narrow(Aneesh B. Singhal, 2006).

Cerebral ischemia manifests itself in two distinctly different pathological areas in the brain referred to as the ischemic core and penumbra. The ischemic core is an area that has the greatest reduction in CBF and undergoes severe irreversible damage. The penumbra is the surrounding area of the ischemic core and is characterized by reduced CBF and O2 metabolism, but an increased O2-extraction fraction, which reflects an attempt to maintain oxygen-dependent high energy metabolism (H. Shi & Liu, 2007)

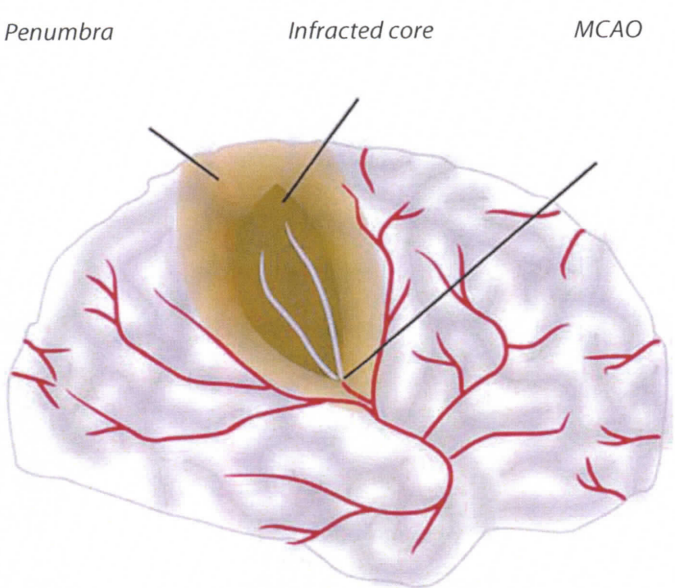

Figure 13 Cerebral ischemia (Mitsios, et al., 2006)

Legend:
Cerebral ischaemia results in rapid cell death in the infracted core within the immediate area of the occluded artery, followed by a less severe infarction in the penumbra in which cells may die over a period of several hours after the insult. MCAO = Middle cerebral artery occlusion.(Mitsios, et al., 2006)

INFLAMMATION

The reduction of cerebral blood flow after stroke elicits a robust inflammatory response in the injured brain, characterized by inflammatory gene expression leading to local activation and release of various cytokines, chemokines, endothelial-leucocyte adhesion molecules and proteolytic enzymes, which propagate inflammatory signals and exacerbate tissue damage for several days after the onset of symptoms . Astrocytes, microglia, leucocytes and endothelial cells (EC) activated by ischaemia produce cytokines. This has been detected in both experimental models of stroke and human patients.

There is evidence for cytokines produced by stimulated macrophages and T cells at the site of the developing cerebral infarction, which then promote migration of leucocytes from the vascular lumen into the brain tissue and regulate inflammatory and immune responses.

Pro-inflammatory cytokines are expressed de novo within the first 2 h after onset of ischaemia and remain elevated for several days after stroke onset, which reflects the intensity of inflammation and its role in tissue damage.(Mitsios, et al., 2006)

Both focal and global brain ischaemia is associated with expression of a number of inflammatory cytokines [interleukin-1 (IL-1) and tumor necrosis factor- (TNF)] and chemokines [IL-8, monocyte chemoattractant protein-1 (MCP-1), RANTES and IP-10], while up regulation of adhesion molecules (ICAM1 and selectins) induces leucocyte recruitment to the vascular endothelium which may affect the survival of damaged neurons (Mitsios, et al., 2006).

Tumor Necrosis Factor Alpha

TNF: A cytotoxic monokine produced by macrophages stimulated with bacterial endotoxin, TNF alpha, participates in inflammation, wound

healing, and remodeling of tissue, its also called *CACHECIN, it was named because of its ability to induce wasting and anemia when administered on a chronic basis to experimental animals. It* can induce septic shock and cachexia. It is a cytokine comprised of 157 amin acid residues, it produced by numerous types of cells including monocytes, macrophages, T lymphocytes, B lymphocytes, NK cells and other types of cells stimulated by endotoxin or other microbial products (Julius M. Cruse, 1995).

Tumor Necrosis Factor- alpha, TNF plays a significant role in brain immune and inflammatory responses, allowing leucocyte adhesion and infiltration into the ischaemic brain. Consequently, agents that suppress TNF production or its actions reduce leucocyte infiltration into ischaemic brain and diminish the extent of tissue loss. Furthermore, TNF activates glial cells, thereby regulating tissue remodeling and scar formation. Anti-inflammatory interventions that limit the degree of damage interfere with nerve regeneration and recovery. In animal models of stroke, ischaemia induces expression of TNF. Increased expression of TNF, mRNA occurs rapidly after middle cerebral artery occlusion (MCAO) in rats at 1–3 h, peaking at 6–12 h postischaemia and subsiding 1–2 days later. TNF was present early in neuronal cells, in infarct and penumbra, and at later time points in macrophages in the infarct. Some studies demonstrated that the size of a brain infarct after MCAO in mice was larger in animals genetically deficient in TNF receptors and the injection of TNF 48 h prior to the occlusion was neuroprotective. There are only a few studies on local inflammation and the development of cerebral ischaemia in humans. There was increased expression of TNF mRNA during the first few hours after MCAO in most animal models. Likewise, in humans, higher cerebrospinal fluid (CSF) and serum levels of TNF were in both infarction volume and mortality. the role of TNF in ischaemic stroke is complex and controversial and probably depends on experimental conditions; most studies suggest a neurotoxic function while some consider it to be neuroprotective. Therefore, a better understanding of TNF downstream observed within the first 6–12 and 24 h after stroke. Interestingly, TNF suppression in mice 30

min after stroke induction ameliorated stroke-related damage at two levels, the primary ischaemic and the secondary inflammatory injury and was accompanied by a decrease signal transduction mechanisms and gene regulation in neuro-inflammation could provide several opportunities for the discovery of novel compounds which may be useful in patients with stroke (Mitsios, et al., 2006).

Neuropeptide Y

Neuropeptide Y (NPY) modulates immune cell distribution,

T helper cell differentiation and natural killer cell activation. Increased NPY immunoreactivity within the perilesional cortex following MCAO or focal excitotoxic damage, in rats has been reported. NPY immunoreactivity was increased at 6 h in the peri-ischaemic region and peaked at around 3 days, decreasing at 10 days. These results suggest that a survival time of 3 days after focal ischaemia is the critical period for examining the relationship between NPY responses and functional

recovery. Peripheral or central administration of NPY impairs reperfusion following MCAO in rat, reduces cerebral blood flow, increases infarct volume and worsens the outcome of focal cerebral ischaemia (Mitsios, et al., 2006).

Excitotoxicity and cell damage

Excitotoxicity is the pathological process by which neurons are damaged and killed by the over activations of receptors for the excitatory neurotransmitter glutamate, such as the NMDA receptor and AMPA receptor, glutamate mediates excitotoxic synaptic transmission via activation of N-methyl- D -aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-propionate (AMPA) or kainite receptors. When glutamate is released from pre-synaptic terminals, it allows Na + and Ca 2+ influx that depolarizes the membrane. While this is vital for neuronal plasticity, additional activation of receptors results in neuronal death. Glutamate is released in an uncontrolled manner in ischaemic areas. The glutamate-calcium cascade induces a necrotic lesion and Ca 2+ -mediated

excitotoxicity plays an important role in brain infarction. Antagonists of glutamate receptors, shown to reduce ischaemic injury in animal models, have been applied in man with little therapeutic beneficial effect. NMDA receptors are highly permeable to Ca 2+ , Na+ , K + and H + cations. Their activation is a primary cause of neuronal death after ischaemia that is accompanied by temporary elevation of extracellular glutamate. Ca 2+ influx mediates NMDA neurotoxicity while Na + influx contributes to swelling of neuronal cell bodies. Normally the free Ca 2+ concentration in the cytoplasm is approximately 1/10,000 of its extracellular concentration. The export of Ca 2+ from neurons into the extracellular environment occurs via processes that are linked to energy utilization. Energy failure in the brain during hypoxia results in a passive efflux of K + from cells, enhancing Ca 2+ entry and release into neurons, the unregulated rise in intracellular cytoplasmic Ca 2+ links glutamate excitotoxicity to biochemical processes resulting in further injury, i.e. oxidative stress. Prolonged elevation of Ca 2+ levels activates Ca 2+ -dependent effectors proteins and enzymes such as endonucleases, phospholipases, lipases, protein kinases and proteases that may damage DNA, lipids and proteins. Identification of the modulators of excitotoxic neuronal death ought to help the development of new approaches to prevent stroke-induced neurological dysfunction (Mitsios, et al., 2006).

Synaptosomal-associated protein 25 (SNAP25) is a neuron-specific protein, primarily localized in nerve endings and axons and involved in synaptic vesicle exocytosis, axonal outgrowth and transmitter release, SNAP25 immunoreactivity was increased in mossy fibers as early as 2, 4 and 7 days after ischaemia and subsided on day 15.(Mitsios, et al., 2006).

NMDA receptor antagonists prevent the expansion of stroke lesions in part by blocking spontaneous and spreading depolarization's of neurons and glia (cortical spreading depression) (Hossmann, 1996)

3.2.4 Mechanisms of ischemic cell death

Ischemic stroke compromises blood flow and energy supply to the brain, which triggers at least five fundamental mechanisms that lead to cell death: excitotoxicity and ionic imbalance, oxidative/nitrative stress, inflammation, apoptosis, and peri-infarct depolarization (Aneesh B. Singhal, 2006).

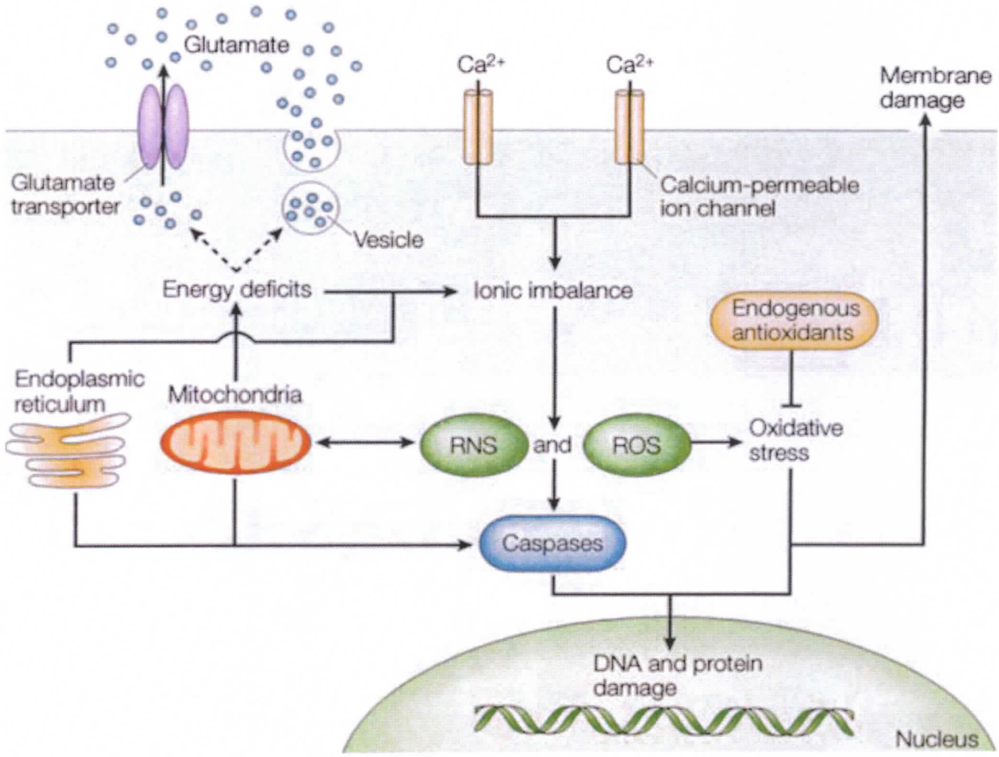

Figure 14: Major pathways of ischemic cells death (Aneesh B. Singhal, 2006)

Legend:
Major pathways implicated in ischemic cell death: excitotoxicity, ionic imbalance, oxidative and nitrative stresses, and apoptotic-like mechanisms. There is extensive interaction and overlap between multiple mediators of cell injury and cell death. After ischemic onset, loss of energy substrates leads to mitochondrial dysfunction and the generation of reactive oxygen species (*ROS*) and reactive nitrogen species (*RNS*).Additionally, energy deficits lead to ionic imbalance, and excitotoxic glutamate efflux and build up of intracellular calcium. Downstream pathways ultimately include direct free radical damage to membrane lipids, cellular proteins, and DNA, as well as calcium-activated proteases, plus caspase cascades that dismantle a wide range of homeostatic, reparative, and cytoskeletal proteins.
(Chawla-Sarkar, et al., 2003)

Ischemic cell death is initiated by changes that result directly from inhibition of oxidative phosphorylation and create an early maelstrom of activity. These changes include decreased pH, decreased ATP, initiation of free radical production by the mitochondrial chain, increased cell Na1 and membrane depolarization as a result of the loss of ATP substrate for the Na1-K1 pump. These lead to secondary changes in ion and chemical concentrations which, in concert with the initiating changes, result in activation of damaging processes. These damaging processes are termed "perpetrators" in this review and are characterized by their abilities to produce long-term changes in macromolecules. Activation of one or more of the perpetrators is considered the key step in necrotic aspects of ischemic cell death (Lipton, 1999)

APOPTOSIS

A variety of key events in apoptosis focus on mitochondria, including the release of caspase activators (such as cytochrome c), changes in electron transport, loss of mitochondrial transmembrane potential, altered cellular oxidation-reduction, and participation of pro- and antiapoptotic Bcl-2 family proteins. The different signals that converge on mitochondria to trigger or inhibit these events and their downstream effects delineate several major pathways in physiological cell death (Green, et al., 1998).

Mitochondria spontaneously released cytochrome c, which activated DEVD-specific caspases, leading to fodrin cleavage and apoptotic nuclear morphology. Bcl-2 acted in situ on mitochondria to prevent the release of cytochrome c and thus caspase activation. During apoptosis in intact cells, cytochrome c translocation was similarly blocked by Bcl-2 but not by a caspase inhibitor, zVAD-fmk. In vitro, exogenous cytochrome c bypassed the inhibitory effect of Bcl-2. Cytochrome c release was unaccompanied by changes in mitochondrial membrane potential. Thus, Bcl-2 acts to inhibit

cytochrome c translocation, thereby blocking caspase activation and the apoptotic process.(Kluck, Bossy-Wetzel, Green, & Newmeyer, 1997).

Apoptosis is the evolutionarily conserved process by which cells die as a result of an internally programmed series of events mediated by a dedicated set of gene products and is associated with condensation of the nucleus and cytoplasm, nuclear fragmentation, and aggregated

condensation of nuclear chromatin, in addition to cell shrinkage, giving way to the development of apoptotic bodies. (Mitsios, et al., 2006)

Cell death following cerebral ischaemia can be described as either necrotic or apoptotic by histological criteria. Necrosis is the predominant mechanism that follows global ischaemia, whereas in milder injury, death may resemble apoptosis. Indeed, data suggesting that apoptosis contributes importantly to brain damage after global or focal ischaemia have expanded within the past 15 years.(Choi, 1996).

Following stroke, there is an early response in gene expression of molecules such as the Bcl-2 family and p53. Then there is a release of proapoptotic molecules such as cytochrome c and apoptosis-inducing factor from mitochondria, leading to activation of caspases and other

genes that augment cell death (Mitsios, et al., 2006)

Despite convergent morphology, it is apparent that apoptosis triggered by different conditions can occur by disparate molecular mechanisms: thymocytes from p53 knock-out mice are resistant to apoptosis induced by radiation, but not by glucocorticoids (Choi, 1996)

p53 and Phosphorylated p53

p53 is expressed in dividing cells following DNA damage; it halts cell division and permits DNA repair, thus preventing the replication of damaged DNA. In several other experimental settings, however, p53 is essential for neuronal death and may participate in neurodegenerative

disorders. p53 induction by oxidative stress/hypoxia results in apoptosis. In addition to elevating the levels of proteins that mediate release of cytochrome c, p53-mediated apoptosis also involves binding to anti-

apoptotic proteins, inhibiting their ability to suppress cytochrome c release.(Mihara, et al., 2003)

Degeneration and death of neurons in stroke involves apoptotic biochemical cascades implicating upstream effectors such as p53. Increased p53 immunostaining has been observed in neurons and glia following focal cerebral ischaemia in the rat.(Li, et al., 1994)

Bcl-2 expression can rescue ciliary neurons deprived of brain-derived neurotrophic factor (BDNF), but not ciliary neurotrophic factor (CNTF)(Choi, 1996).

The Bcl-2 gene family, including anti-apoptotic and pro-apoptotic counter molecules, is a fundamental gene group in cell death-signaling cascades. The relative amounts of active anti- and pro-apoptotic Bcl-2 family proteins determine the sensitivity or resistance of cells to apoptosis. The chief function of Bcl-2 family proteins is to regulate the release of cytochrome c and other pro-apoptotic proteins from mitochondria.(Mitsios, et al., 2006).

Caspase-3 and Active Caspase-3

Activation of caspases by cytochrome c appears to be an integral part of apoptotic cell death. Caspase-3 can be activated by caspase-8 and caspase-9 and is a well-characterized pro-apoptotic executioner, involved in proteolytic processes in apoptotic cells.

Biochemical and immunohistochemical studies have demonstrated expression and activation of caspase-3 after stroke although conflicting data with respect to the presence of caspase-3 under ischaemic conditions exist.(Mitsios, et al., 2006).

Neuronal caspase-3 levels increased 16–24 h after permanent MCAO in rat(Krupinski, Lopez, Marti, & Ferrer, 2000).

Neuronal death induced by hypoxia-ischemia has seemed for years an open-and-shut case for necrosis Wyllie and colleagues, used hypoxia-ischemia as an example of a 'violent' environmental perturbation capable of producing necrosis. The implication of excitotoxicity as an important component of ischemic neuronal death over the past decade has further

strengthened this notion, as the central features of excitotoxicity would seem to epitomize membrane failure and necrosis. Overstimulation of neuronal glutamate receptors typically leads to rapid, dramatic cell body and dendrite swelling, mediated by excessive Na + and Ca 2+ entry through glutamate receptor gated channels, associated with CI- and water influx(Choi, 1996).

Free radicals play a significant role in cell signaling and the induction and activation of multiple genes. For example, there is growing evidence that free radicals influence the action of proteases at multiple levels, including transcription and processing of mRNA and activation of latent proteases, induction of hypoxia inducible factor 1, and caspase-involved apoptosis.(H. Shi & Liu, 2007)

CHANGES OF TISSUE OXYGEN AFTER CEREBRAL ISCHEMIA & REPERFUSION

Although the cerebral oxygen level is a critical issue in cerebral ischemia, monitoring oxygen levels in cerebral tissue *in vivo* and in real-time remains very challenging technically, especially in deeper tissue when repetitive measurements are needed. Several techniques are available that can be used to measure tissue oxygen, including Clark-type electrodes, fluorescence quenching, phosphorescence quenching, near infrared spectroscopy, MRI, and electron paramagnetic resonance (EPR) oximetry. However, each of these techniques has its own particular advantages and limitations. For example, Clark-type electrode techniques cause traumatic lesions that do not heal when the electrode is inserted in to the brain. Optical measuring techniques for brain tissue pO2 have the limitation of measurement depth (H. Shi & Liu, 2007)

Neuroprotective

SPECIFIC PROTEINS are expected to be released from neurons and their processes following central nervous system (CNS) injury and disease. An

assay that could reliably quantify the levels of these released proteins might provide useful information about the degree of neuronal injury occurring as a result of damage and disease states, and would be particularly useful if such proteins could be detected in blood.(K. J. Anderson, et al., 2008)

Neuroprotection can be defined as the protection of cell bodies and neuronal and glial processes by strategies that impede the development of irreversible ischemic injury by effects on the cellular processes involved. Neuroprotection can be achieved using pharmaceutical or physiological therapies that directly inhibit the biochemical, metabolic, and cellular consequences of ischemic injury, or by using indirect approaches such as t-PA which was mentioned earlier and mechanical devices to restore tissue perfusion (Aneesh B. Singhal, 2006).

One of the first groups of neuroprotective genes expressed after stroke is the heat shock protein family (HSPs), which acts to prevent aggregation of denatured proteins and aid their refolding to the correct tertiary structure. Its an antibodies of the IgM, IgG classes specific for a 73 kD chaperonin that belongs to the hsp70 family. (Julius M. Cruse, 1995)

HSP70 is one of the major inducible stress proteins upregulated in response to ischaemia. Its mRNA is usually expressed within 1–2 h and then down regulated by 1–2 days. HSP70 may be a marker for neuroprotection in the early stages of ischaemic stroke and many mechanisms have been suggested for HSP70 protection from cerebral ischaemia, including defense against apoptotic and necrotic cell death. (Mitsios, et al., 2006).

POST-STROKE RISKS "COMPLICATIONS"

After a stroke patients have several risks factor such as. Recurrent of vascular events post stroke are well documented and studies have suggested that rates higher than previously expected; a 5-year stroke recurrence rate of up to 16% and a cumulative 10-years risk for vascular events of 44.1% have been quoted. (McManus, et al., 2009)

Patients have a higher risk of experiencing a fall. Falls are one of most common complications of rehabilitation in the patient's populations. According to the study which was done in Poland by (Czernuszenko & Czlonkowska, 2009), the study was done on 1155 patients 56% were men, the study was from (1-74 days), total of 252 falls were recorded for 189 (16.3%) patients and 45 patients experiences 108 repeated falls. The incidence of falls was 7.6/1000 patients a days. Falls occurred in 16% of patients during post-stroke inpatient's rehabilitation, but only 1.2% of falls (1.6% of patients who fall) result in fractures. Increase risk factor of all falls is strongly associated with severe baseline stroke-related disability, short time (<12 weeks) after stroke onset and greater improvement in functional status during rehabilitation. The study showed a severe risk related in early period after stroke are prone to falls during rehabilitation, multiple falls are most frequent in patients over 65 years age. (Czernuszenko & Czlonkowska, 2009). According to the randomized study which was done by (McManus, et al., 2009), the study appears to be one of a small number carried out showing long-term outcomes in post stroke patients is disappointing, it seems that brief intervention with education and counseling had no long term benefit on behavior modification or risk factor control.

(Kluding & Gajewski, 2009) strength deficits in the hemiparetic lower extremity should be an important target for clinical interventions to improve function in people with chronic stroke.

4 Methodology

ADVISORY GROUP

We identified technical experts to assist us in formulating the research questions and identifying relevant databases for the literature search. The expert panelists included a neurologist specializing in stroke, a physician with an HBOT practice. Throughout the project period, we consulted individual members on issues that arose in the course of identifying and reviewing the literature.

Literature Search, Study Selection, and Data Extraction

We searched a broad range of databases to identify published and unpublished studies of the effectiveness and harms of HBOT in patients with brain injury, and stroke, neurological disorders and other internal disorders

We selected and extracted recent articles from 1962- 2009 that we felt to be of relevance or interesting as well as choosing topics that we were aware of being potentially important.

Each database was searched from its starting date to August 2008. The databases searched were:

- MEDLINE®
- Pre MEDLINE
- EMBASE
- ProQuest
- Oxford journal
- American heart association
- Cochrane Database of Systematic Reviews

- Cochrane Controlled Trials Register
- DARE (Database of Abstracts of Reviews of Effectiveness)
- CINAHL (Cumulative Index to Nursing & Allied Health)
- MANTIS (Manual, Alternative and Natural Therapy)
- Health Technology Assessment Database
- The New England Journal of Medicine
- The Undersea & Hyperbaric Medical Society: a large bibliographic database (30,000 records), http://www.uhms.org/library.htm
- The Database of Randomized Controlled Trials In Hyperbaric Medicine,http://hboevidence.com/
- European Underwater and Baromedical Society, http://www.eubs.org/
- International Congress on Hyperbaric Medicine, http://www.ichm.net/

- TEAG members identified the following additional databases as potential sources of other material that may not be

indexed in other electronic databases:

- European Underwater and Baromedical Society
- International Congress on Hyperbaric Medicine
- National Baromedical Services, Inc.
- Update literature searching of the electronic databases

MEDLINE, PreMEDLINE, EMBASE, CINAHL, the Cochrane Library, and the Health Technology Assessment Database was completed on February 26, 2009, using the same search strategy as used for the initial searches. Eight additional references submitted by a peer reviewer were added in March 2009.

The references of all included papers were hand searched. In

addition, we review independently and conducted hand searches of the references from the (Eric P. Kindwall, (September 3, 2004); Frank H. Netter, 2005; J.C.E.UNDERWOOD, (1992); Jain, 2004) We reviewed as well exclude independently and assessed each title and abstract located through the literature searches for relevance to the review, based on the intervention, population, outcome, and study design criteria. The full-text articles, reports, or meeting abstracts that met the criteria listed above were retrieved and reviewed independently by one reviewer who reapplied the eligibility criteria. Disagreements were resolved through consensus. One reviewer performed extraction of data from studies.

SCOPE

To identify the patient groups, interventions, and outcomes that should be included in the review, we read background material from diverse sources including textbooks, government reports, proceedings of scientific meetings, and Web sites. We also conducted focus group and interviews to improve our understanding of the clinical logic underlying the rationale for the use of HBOT. In the focus group, we identified outcomes of treatment with HBOT that are important to patients and examined whether patients, who have experience with HBOT value certain outcomes differently. A broader goal of the focus was to better understand the main pathophysiologic reactions that brain cells undergo during ischemic brain attack. And the free radicals in the human body specifically in mitochondria. By reviewing the fundamentals of stroke we aim to achieve a clear view on the subject in order to recognize the therapeutic specificity that hyperbaric oxygen therapy (HBOT) offers. We also took a closer look on the basics of HBO, its history, physiological aspects, indications, the principals that accompany it.

VALIDITY OF EVIDENCE OF THE DATA

The analyses of individual studies reviewed for quality and consistent evidence for each key question is based on the sufficient Evidence , Validity of the information also regarded Author's opinion and advices due to there experience's in the case of the hyperbaric oxygenation we also included arguments of these authors about hyperbaric oxygenation and oxidative stress. The quality of all trials in the review was assessed using a list of items indicating components of internal validity. We modified the standard checklists to address issues of particular importance in studies of HBOT. For randomized controlled trials (RCTs) and nonrandomized controlled trials (NRCTs),

Each study was assigned an overall rating (good, fair or poor) according to the US Preventive Services Task Force methods.

Good:

Comparable groups assembled initially (adequate randomization and concealment, and potential confounders distributed equally among groups) and maintained throughout the study; follow up at least 80 percent; reliable and valid measurement instruments applied equally to the groups; outcome assessment masked; interventions defined clearly; all important outcomes considered; appropriate attention to confounders in analysis; for RCTs, intention-to-treat analysis.

Fair:

Generally comparable groups assembled initially (inadequate or unstated randomization and concealment methods) but some question remains whether some (although not major) differences occurred with follow-up; measurement instruments acceptable (although not the best) and generally applied equally; outcome assessment masked; some, but not all important outcomes considered; appropriate attention to some, but not all potential confounders; for RCTs, intention-to-treat analysis.

Poor:

Groups assembled initially not close to being comparable or not maintained throughout the study; measurement instruments unreliable or invalid or not applied equally among groups; outcome assessment not masked; key confounders given little or no attention; for RCTs, no intention-to-treat analysis.

The discussion of results and conclusions in this report is based on the analysis of information extracted from the above mentioned text books as well as good- and fair-quality studies. Results of good-quality studies have a high likelihood of being both valid and reliable. Fair-quality studies have important but not fatal flaws in their design or conduct. The category of fair is broad, with some studies that are probably valid and others that are unlikely to be valid, depending on the specific flaws found and their severity. The inadequacies found in poor-quality studies make the results unreliable.

DATA EXTRACTION SYNTHESIS

We extracted data from literatures, articles and studies which is in the point of the aims of this work and critically reviewed to be afterward synthesized to draw together a symmetrical line in the thesis review to meet the aims and the Scope and Key questions, an analysis of whether the body of evidence is sufficient to provide a clear answer to the key questions' was looked.

Evidence

The analyses of individual studies reviewed for quality and consistent evidence for each key question is based on the internal and external validity. An analysis of whether the body of evidence is sufficient to provide a clear answer to the key questions was undertaken.

5 The analyses of Data and Discussion

Findings

Brain Injury

For traumatic brain injury, some trial studies were provided fair evidence that HBOT might reduce mortality or the duration of coma in severely injured TBI (traumatic brain injuries) patients. However, HBOT also increased the chance of a poor functional outcome. (G. L. Rockswold & Ford, 1985; G. L. Rockswold, Ford, Anderson, Bergman, & Sherman, 1992; S. B. Rockswold, et al., 2007)

The quality of the controlled trials was fair, meaning that deficiencies in the design add to uncertainty about the validity of results.

(Lin, et al., 2008) did a study group randomly included 22 patients who received HBOT after the patients' condition stabilization, and the other 22 corresponding condition patients were assigned into the matched control group who were not treated with HBOT. The clinical conditions of the patients were evaluated with the Glasgow Coma Scale (GCS) and Glasgow Outcome Scale (GOS) before and 3 to 6 months after HBOT. The GCS of the HBOT group was improved from 11.1 to 13.5 in average, and from 10.4 to 11.5 ($p < 0.05$) for control group. Among those patients with GOS = 4 before the HBOT, significant GOS improvement was observed in the HBOT group 6 months after HBOT. *Based on this study, HBOT can provide some benefits for the subacute TBI patients with minimal adverse side effects.*

[Palzur and colleague's (Palzur, Zaaroor, Vlodavsky, Milman, & Soustiel, 2008)] their recent experimental data have shown that hyperbaric oxygen therapy (HBOT) was associated increased Bcl-2 expression at the injury site that correlated with reduced apoptosis. We hypothesized that HBOT mediated enhancement of Bcl-2 expression and increased intracellular oxygen bioavailability may both contribute to preserve mitochondrial integrity and reduce the activation of the mitochondrial pathway of apoptosis. For this purpose, a cortical lesion was created in the parietal

cortex of Sprague-Dawley rats by dynamic cortical deformation (DCD) and outcome measures in non-treated animals were compared with that of HBOT treated rats. Morphological analysis showed a profound reduction in neuronal counts in the perilesional area and a marked rarefaction of the density of the axonal-dendritic network. In treated animals, however, there was a significant attenuation of the impact of DCD over perilesional neurons, characterized by significantly higher cell counts and denser axonal network. In mitochondria isolated from injured brain tissue, there was a profound loss of mitochondrial transmembrane potential (Deltapsi(M)) that proved to be substantially reversed by HBOT. This finding correlated with a significant reduction of caspases 3 and 9 activation in HBOT treated animals but not of caspase 8, indicating a selective effect over the intrinsic pathway of apoptosis. *All together, our results indicate that the neuroprotective effect of HBOT may represent the consequence of preserved mitochondrial integrity and subsequent inhibition of the mPTP and reduction of the mitochondrial pathway of apoptosis.*

[Shi and colleagues (X. Y. Shi, et al., 2006)] studied three hundred and ten patients with neuropsychiatric disorders arising from traumatic brain injury were treated twice with hyperbaric oxygen. Cerebral single photon emissions computed tomography (SPECT) images and computed tomography scans (CT) before and after hyperbaric oxygen treatment, were compared. RESULTS: Before treatment, the proportion of abnormal cerebral changes detected by SPECT was 81.3% but only 15.2% by CT. After HBO treatment, 70.3% of SPECT scans showed no abnormalities and these patients were clinically improved. Treatment improved regional cerebral blood flow. CONCLUSION: *SPECT was much more sensitive than CT in the diagnosis of neuropsychiatric disorders following hyperbaric oxygen treatment of neuropsychiatric disorders arising from traumatic brain injury.*

[Voldavsky and colleagues, (Vlodavsky, Palzur, & Soustiel, 2006)] found in correlation with secondary cell death in the rat model of dynamic cortical deformation (DCD). Twenty animals underwent DCD with subsequent

HBOT (2.8 ATA, two sessions of 45 min each); 10 animals: DCD and normobaric oxygenation (1 ATA), 10 animals: not treated after DCD. Cell death was evaluated by TUNEL. Neutrophils were revealed by myeloperoxidase staining. Immunohistochemical staining for MMP-2 and -9 and tissue inhibitors of MMP-1 (TIMP-1) and -2 was also performed and the results were quantitatively evaluated by image analysis. In the animals treated by HBOT, a significant decrease in the number of TUNEL-positive cells and neutrophilic inflammatory infiltration was seen in comparison with non-treated animals and those treated by normobaric oxygen. The expression of MMP-9 was also significantly lower in the treated group. Staining for MMP-2 and TIMP-2 did not change significantly. Our results demonstrate that HBOT decreased the extent of secondary cell death and reactive neuroinflammation in the TBI model. The decline of MMP-9 expression after HBOT may also contribute to protection of brain tissue in the perilesional area. Further research should be centered on the evaluation of long-term functional and morphological results of HBOT.

(S. B. Rockswold, et al., 2001) studied oxygen (100% O2, 1.5 atm absolute) was delivered to 37 patients in a hyperbaric chamber for 60 minutes every 24 hours (maximum of seven treatments/patient). Cerebral blood flow, arteriovenous oxygen difference (AVDO2), cerebral metabolic rate of oxygen (CMRO2), ventricular cerebrospinal fluid (CSF) lactate, and ICP values were obtained 1 hour before and 1 hour and 6 hours after a session in an HBO chamber. Patients were assigned to one of three categories according to whether they had reduced, normal, or raised CBF before HBO. In patients in whom CBF levels were reduced before HBO sessions, both CBF and CMRO2 levels were raised 1 hour and 6 hours after *HBO* ($p < 0.05$). In patients in whom CBF levels were normal before HBO sessions, both CBF and CMRO2 levels were increased at 1 hour ($p < 0.05$), but were decreased by 6 hours after HBO. Cerebral blood flow was reduced 1 hour and 6 hours after HBO ($p < 0.05$), but CMRO2 was unchanged in patients who had exhibited a raised CBF before an HBO session. In all

patients AVDO2 remained constant both before and after HBO. Levels of CSF lactate were consistently decreased 1 hour and 6 hours after HBO, regardless of the patient's CBF category before undergoing HBO ($p < 0.05$). Intracranial pressure values higher than 15 mm Hg before HBO were decreased 1 hour and 6 hours after HBO ($p < 0.05$). The effects of each HBO treatment did not last until the next session in the hyperbaric chamber. CONCLUSIONS: *The increased CMRO2 and decreased CSF lactate levels after treatment indicate that HBO may improve aerobic metabolism in severely brain-injured patients. This is the first study to demonstrate a prolonged effect of HBO treatment on CBF and cerebral metabolism. On the basis of their data the authors assert that shorter, more frequent exposure to HBO may optimize treatment.*

(Chang, et al., 2000) experimented on adult male Sprague–Dawley rats weighing 250 –300 g were used in these experiments, Animals were randomly assigned to HBO (n= 10) , HBP (n =10) , or NAP treatment condition (n =5) . All tests were run blinded, and the animal codes were revealed only at the end of the behavioral and histological, The MCA embolic technique involved insertion of a neurofilament through the carotid artery to reach the junction of the MCA, thus blocking the blood flow from the common carotid artery. Animals exposed to HBO either immediately after or 60 min after MCAo showed a significant amelioration of asymmetrical behaviors as revealed by the elevated body swing test (65 and 55% contralateral biased swing, respectively) compared to animals exposed to NAP (90%) animals exposed to HBO either immediately after or 60 min after MCAo manifested a significant reduction ($P < 0.05$) in infarction volume compared to animals exposed to NAP. Animals exposed to HBP immediately after MCAo also displayed significant reduction in infarction volume ($p < 0.05$), but those exposed to HBP 60 min after MCAo only showed trend ($p=0.06$) toward decreased infarction compared to animals exposed to NAP. Animals exposed to HBO either immediately after or 60 min after MCAo showed a significantly reduction ($p< 0.05$) in incidence of infarction (20 and 16.7% incidence, respectably) compared to

animal exposed to NAP (100%). He concluded that= The present observation suggest that HBO attenuated ischemia-induced brain damage, and behavioral dysfunctions, while HBO and HBP did not significantly differed in some parameters of "protection" examined here. HBO produced more consistent and effective than normal baric therapy. The main findings in this study that HBO appears more effective than HBP. Although the animals exposed to HBP have significant reduction in cerebral infarction they did not show consistent recovery of motor deficits.

The evidence for use of HBOT in other types of brain injury is conclusive. Some fair-quality studies were found. But to be able to confirm it we have to do more randomized and qualitative studies.

Stroke

Although a large number of studies address HBOT for the treatment of stroke, the evidence is insufficient to determine whether HBOT reduces mortality in any subgroup of stroke patients because no controlled trial assessed was designed to assess mortality.

Among controlled trials, the evidence about morbidity is conflicting. The three best-quality trials found no difference in neurological measures in patients treated with HBOT versus patients treated with pressurized room air.

Two other controlled trials, one randomized and one nonrandomized, found that HBOT improved neurological outcomes on some measures. However, both were rated poor-quality.

Most observational studies reported favorable, and sometimes dramatic, results, but failed to prove that these results can be attributed to HBOT. For example, one retrospective study found better mortality rates in patients who received HBOT than a comparison group of patients from a different hospital who did not. The study did not provide information on mortality rates from other causes in each hospital; this information would have made it easier to judge whether the improved survival was due to HBOT

or to differences in overall quality of care at the HBOT hospital.

The observational studies of HBOT provided insufficient evidence to establish a clear relationship between

physiologic changes after HBOT sessions and measures of clinical improvement. Few studies established that patients were stable at baseline.

There was a study and the result was decreased in oxygen consumption occurred in brain slices when exposed to high HBO pressure. (Mulkey, Henderson, Olson, Putnam, & Dean, 2001)

Adverse Events

Evidence about the type, frequency, and severity of adverse events in actual practice is inadequate. Reporting of adverse effects was limited, and no study was designed specifically to assess adverse effects.

The few data that are available from controlled trials and cohort studies of TBI suggest that the risk of seizure may be higher in patients with brain injuries treated with HBOT.

No study of HBOT for brain injury, cerebral palsy, or stroke has been designed to identify the chronic neurologic complications.

Pulmonary complications were relatively common in the trials of brain-injured patients. There are no reliable data on the incidence of aspiration in children treated for cerebral palsy with hyperbaric oxygen.

Ear problems are a known potential adverse effect of HBOT. While ear problems were reported in brain injury, cerebral palsy, and stroke studies the incidence, severity and effect on outcome are not clear. However, the rates

reported among cerebral palsy patients were higher (up to 47 percent experiencing a problem) than reported with brain injury or stroke. However, the data in brain injury are limited by the use of prophylactic myringotomies.

Supplemental Qualitative Analysis

Opinions about the frequency and severity of risks of HBOT vary widely.

Several participants emphasized the importance of continued treatments to maximize results.

Patients and caregivers value any degree of benefit from HBOT highly. An improvement that may appear small on a standard measure of motor, language, or cognitive function can have a very large impact on caregiver burden and quality of life.

Better outcome measures. In describing the course of their patients, experienced clinicians who use HBOT to treat patients with brain injury, cerebral palsy, and stroke refer to improvements that may be ignored in standardized measures of motor and neuro-cognitive dysfunction. These measures do not seem to capture the impact of the changes that clinicians and parents perceive. Caregivers' perceptions should be given more weight in evaluating the significance of objective improvements in a patient's function. Unfortunately, studies have not consistently measured caregiver burden, or have assessed it only by self-report. Studies in which the caregivers' burden was directly observed would provide much stronger evidence than is currently available about treatment outcome.

Adverse events. Uncertainty about the frequency and severity of serious adverse events underlies much of the controversy about HBOT. The case against HBOT is based on

The reasoning that, because HBOT may be harmful, it must be held to the highest standard of proof. A corollary is that, if HBOT can be shown to be as safe as its supporters believe it to be, the standard of proof of its efficacy can be lowered.

Good-quality studies of adverse effects are designed to assess harms that may not be known or even suspected. The most common strategy is to use a standard template of several dozen potential adverse effects affecting each organ system. Other characteristics of a good study of adverse events are a clear description of patient selection factors, independent assessment

of events by a neutral observer, and the use of measures for the severity (rather than just the occurrence) of each event.

Unwillingness to be in a placebo group. The issue of placebo groups has been the subject of a great deal of debate. Participants on both sides make the assumption that an "evidence-based" approach implies devotion to double blind, placebo-controlled trials without regard to practical or ethical Considerations. This assumption is false. Double blind, placebo-controlled trials are the "gold standard" for government regulators overseeing the approval of new pharmaceuticals, but not for clinical decision making or for insurance coverage decisions. Evidence-based clinical decisions rely more heavily on comparisons of a treatment to other potentially effective therapies than to placebos.

Several alternatives to the double blind, placebo-controlled trial can be used to examine effectiveness. One approach is to compare immediate to delayed treatment with HBOT, as was done in the Cornell trial. Another is to design a trial in which patients are randomly assigned to several alternative HBOT.

Regimens. Because of uncertainty about the dosage and duration of treatment, such a trial would be preferable to a trial that offered a choice between one particular regimen and no treatment at all. It is also easier to incorporate a sham therapy arm in such a trial: patients may be more willing to enter a trial if they have a 10 percent or 20 percent chance of being assigned to sham treatment instead of a 50 percent chance. Other alternatives to a placebo include conventional physical, occupational, and recreational therapy, or another alternative therapy, such as patterning.

The Canadian trial of HBOT for cerebral palsy has important implications for the design of future research. In the trial there was a clinically significant benefit in the control group. Debate about the trial centers largely on how the response in the control group should be interpreted. The trial investigators believe that the beneficial effect was the result of the psychological effect of participating in the trial and extra.

Attention paid the children in and out of the hyperbaric chamber. Alternatively, the slightly pressurized air (that is, "mild" hyperbaric oxygen) may have caused the improvement. A third possibility is that the slightly increased oxygen concentration, not the pressure per se, was responsible for the benefit.

A trial that could sort out which of these explanations was true would have a major impact on clinical practice. Such a trial might compare (1) room air under slightly elevated pressure, delivered in a hyperbaric chamber, to (2) elevated oxygen concentration alone, delivered in a hyperbaric chamber,

And to (3) an equal amount of time in a hyperbaric chamber, with room air at atmospheric pressure. From the perspective of a neutral observer, the third group is not a "sham" but rather an attempt to isolate the effect of the social and psychological intervention cited by the Canadian investigators.

In addition to needing improved design, future trials of HBOT need better reporting. This would aid interpretation and the application of the research results. Two types of information are essential: a clear description of the research design, particularly of the control and comparison groups, and

A detailed description of the patient sample. It is frequently difficult to tell from published studies how comparable the patient populations are, not only demographically but also clinically, in order to interpret the diagnosis and prognosis.

HBOT for brain injury is not likely to gain acceptance in routine clinical use until a clinical method of assessing its effectiveness in the individual patient is validated. Specifically, the diagnostic value of SPECT scans and of other intermediate indicators of the effects of HBOT should be examined in good quality studies. Like all other diagnostic tests, SPECT scans have a measurable false positive and false negative rate in relation to clinical outcomes. Controlled trials are not needed as the ideal study design to measure the accuracy of a diagnostic test. Rather, a longitudinal cohort study in which all patients undergo scans as well as standardized follow-up tests would be a feasible and ideal approach.

Synthesis of Results

Results of data extraction and assessment of study validity are presented bellow as a narrative description.

6 Results

In this review, we sought to analyze the effect of HBOT in acute ischemic stroke by answering the question: the overall benefits regarding hyperbaric oxygen therapy after acute ischemic stroke.

What is the relation between HBOT and free radicals "Oxidative stress"?

Hyperbaric oxygenation has a several disadvantages, overall main disadvantage is the free radicals "oxidative stress" and that the main debate on now a day science. The free radical are produced normally in the mitochondria under normal circumstances. And the other main disadvantage is the possibility of oxygen toxicity.

Under normal circumstances, the rate of generation of superoxide from mitochondria is rather low and dose little damage, simply because it is efficiently removed by the superoxide dismutase's. Circumstance's can arise for a variety of reasons (e.g. ingested chemicals that act as radical amplifiers, medically applied high concentrations of oxygen, or during periods of reperfusion of tissue with oxygen following ischemia).(Raha & Robinson, 2000)

Alcohol-induced increases in the activity of the enzyme cytochrome P450 2E1 (CYP2E1), which as described in the section "Systems Producing ROS" metabolizes alcohol and other molecules and generates ROS in the process. Alcohol-induced increases in the levels of free iron in the cell (i.e: Iron that is not bound to various proteins), which can promote ROS Generation, as described in the section "Role of Metals."(Wu & Cederbaum, 2003)

Some mechanisms that related with CNS toxicity have been studied. The

production of free radical and their toxic effect on brain, the disturbance of neurotransmitter, endothelium derived relaxing factor (EDRF) and its syntheses, constriction and/or dilation of blood vessel in brain and metabolic dysfunction which was done by [Demchenko et al 2000 (W. Liu, et al., 2008)] HBO exposure can induce a proportional increase in the rate of formation of reactive oxygen species (ROS) such as superoxide anion, singlet oxygen, and hydroxyl radical [Dircks and Faiman 1982; (W. Liu, et al., 2008)], direct or indirect evidence for free radical generation in HBO exposures were provided through several methods. ROS may evoke seizures by various mechanism including excitotoxicity, metabolic dysfunction, and disturbance of intracellular homeostasis of calcium.

Recent studies have implicated nitric oxide (NO) as a mediator of CNS toxicity. NO is formed from agrinine by the action of one of three different nitric oxide syntheses (NOS) isozymes which are two calcium-dependent form, neuronal (nNOS) and endothelial (eNOS), and one calcium independent form (iNOS).

Two lines of evidence supported the role of NO in epileptogenesis:

1. NO concentration in brain increases during exposure to HBO.
2. NOS, inhibitors, which block NO production, can protect rats and mice against hyperoxic seizures (N. Bitterman & Bitterman, 1998; M. Chavko, Braisted, Outsa, & Harabin, 1998).

   (W. Liu, et al., 2008) repetitive HBO exposure (preconditioning) could affect the sensitivity of mice to convulsions during subsequent exposure to HBO.

   [Claudia and colleagues,(Claudia Dennog, Andreas Hartmann, Gunter Frey, & Gunter Speit, 1996)] the study we demonstrated for the first time the induction of oxidative DNA damage by HBO. A clear reproducible genotoxic effect was seen with the comet assay immediately after HBO exposure (3x20 min at 2.5 ATA) as used therapeutically.

Dose the Dosage and exposure time in the chamber enhance the production of free radicals?

Our result demonstrated that HBOT protect motor cortex and spinal cord mitochondria from complex IV dysfunction in the Wobbler mouse. A number of studies suggest that pronounced levels of free radicals are only observed at either high pressure or long HBOT exposure. Oxidative stress and resultant tissue damage are the hallmark of cell death, there is increasing production and/or ineffective scavenging of such reactive oxygen species may play a crucial role in determining tissue injury. The level of intermediate reduction products of oxygen metabolism (Yasar, et al., 2003).

There were some studies confirming that the dosage and the period of exposure might increase the risk of oxidative stress, but further qualitative studies have to confirm our findings.

Dose antioxidant and other medicaments maintain the production of free radicals?

As we were searching through the data regarding a HBOT and antioxidants or medications, we were not able to find qualitative studies and we mainly found one study, which was a poor one, and the question wasn't answered.

7 Conclusion

As we went through literatures including books and studies including different kind of controlled and uncontrolled studies. *(See appendix)*

We determined whether the benefits of HBOT outweigh the potential harms, there was a possibility that the patient would have oxygen toxicity or free radical productions.

The main conclusion that the studies were done either with a high dosage of the HBOT, or a long duration, repetition of the therapy was included in the same day which lead by the end of the therapy to oxygen toxicity or the production of free radical were present.

No controlled trial of HBOT was designed to measure mortality in stroke patients, and the best studies found somewhat improvement in neurological outcomes.

Evidence about the type, frequency, and severity of adverse events in actual practice is inadequate.

Reporting of adverse effects was limited, and no study was designed specifically to assess adverse effects. Evidence from well-conducted clinical studies is limited.

The balance of benefits and harms of HBOT for brain injury, cerebral palsy, or stroke has not been adequately studied.

We concluded that the HBOT has a promising great future for the benefit of patients, health care and families of the patients. Diabetic patient and post-cosmetics surgeries should promising prognosis. More studies have to be done, to confirm the proper depth and duration of the therapy to confirm its best result and prognosis in stroke, cerebral edema. *(See discussion and future research)*

RECOMMEDATION FOR THE FUTURE RESEARCH

A proper study which well involve giving the patient appropriate medications that influence the improvement of the neurons and brain activity, there is a lack of studies which patient were given medicaments, there is no trails were given with medicaments to any of the articles we went through beside vitamin supplement's which were taking during the therapy in studies, *(see the article review and appendix).*

Strategies can be developed to conduct good-quality studies to overcome each of these barriers. **Dose and duration of treatment.** Oxygen, the "active ingredient" in HBOT, is fundamentally a drug. As for any drug, dose and duration of treatment must be determined carefully designed dose-ranging studies before definitive studies demonstrating clinical efficacy can be started.

Good-quality dose-ranging studies of HBOT for brain injury can be done, based on the model used by pharmaceutical manufacturers and the FDA.

It is likely that the dosage of HBOT needs to be individualized based on the patient's age, clinical condition, and other factors.

This is the case for many other drugs and does not pose an insurmountable barrier to designing dose finding trials. In fact, the need to individualize therapy makes it essential to base the design of long-term studies of clinical outcomes on the results of dose-ranging studies.

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Appendix

List of appendix:

1- Animal experimental studies
2- Uncontrolled trails
3- Controlled trails
4- Random studies

Appendix 1: Animal experiments

Authors & year	Animal Model	Technique of ischemia/anoxia	Treatment protocols	Measurement and results
Smith et al (1961)	Dogs	CO inhalation	HBO 2ATA, 100% O2 controls, no treatment	Neurological recovery facilitated; cortical vessel diameter, no change if CO2 is 40 mm
Whalen et al (1966)	Dogs	Induced cardiac fibrillation	HBO 3 ATA, 100% oxygen controls, no treatment	Prolonged the period from fibrillation to cessation of EEG activity; cerebral protection
Corkill et al (1986)	Gerbils	Carotid ligation	HBO 1.5 to 2 ATA with 100% O2; control air	Calorimetric videodensimetric estimation of cerebral ischemia through intact cranium; best results obtained in HBO groups
Kawamura et al (1990)	Rats	Middle cerebral artery occlusion for 4 h	HBO at 2 ATA 100% O2, for 30 min. treatment given between 2.5-3.5 h following ischemic insult	HBO reduce ischemic neuronal injury and brain edema
Authors & year	**Animal Model**	**Technique of ischemia/anoxia**	**Treatment protocols**	**Measurement and results**
Yatsuzuka	Dogs	Complete	HBO at 2	CBF, EEG and

(1991)		cerebral ischemia for 18 min	ATA for 170 min	indicators of oxidative stress, HBO reduced brain damage without increasing oxidative stress.
Takahashi et al (1992)	Cats	Global ischemia induced by occlusion of ascending aorta and caval veins for 15 min.	HBO at 3 ATA 100% O2 for 1 h at 3, 24 & 29 h after ischemia, controls; room air	Survival rats were 30% in control group Vs. 70% in-group treated with HBO. Neurological recovery with slight disability was in 1 of 10 control animals vs. 6 out of 9 animals in the HBO-treated group
Sunami et al (2000)	Rats	Ligation of the right MCA and right common carotid artery	HBO (3ATA) 2 h, initiated 10 min after onset of ischemia	Reduced infarct volume by increasing oxygen supply to the ischemic periphery without aggravating lipid peroxidation
Badr et al (2000)	Freely moving rats	MCA occlusion. After 2 h of occlusion the suture was removed and reperfusion was allowed	HBO (3ATA, 1 h), 100 % O2	Neuroprotective effect as HBO normalized brain energy metabolites and excitatory amino acids disturbances that occur during cerebral ischemia.
Authors &	**Animal**	**Technique of**	**Treatment**	**Measurement and**

year	Model	ischemia/anoxia	protocols	results
Hjelde et al (2000)	Wistar rats	Right middle cerebral occluded for 4 h	HBO(2ATA, 100% O2 for 230 min)	HBO did not reduce tissue damage
Yin et al (2002)	Rata	Transient middle cerebral artery occlusion with focal ischemia	HBO (3ATA,100% O2 for 1 h) given 6 h after perfusion	HBO within 6 h reduced infarction, there was inhibition of COX-2-over-expression in cerebral cortex accompanying the neuroprotective effect

Appendix 2: Uncontrolled Trails

Authors & Year	Diagnosis	No. of patients	HBO Protocol	Method of Evaluation & Result
Ingever and Lassen (1965)	Focal cerebral ischemia	4 Single treatment	2 2.5 ATA 1.5 to 2.5 h	Improvement in 3 patients; clinical EEG
Saltzman et al (1966)	Acute cerebrovascular insufficiency	25	2-3 ATA, 2 h/day; 15 treatment	8 improved dramatically; 5 improved during but regressed later; 12 did not improve
Neubauer and End (1980)	Stroke; acute and chronic cases`	122	1.5-2 ATA, 1 h/day within 1 h of onset of stroke	Total hospitalization stay of treated patients was 177 days (287 days in untreated patients);65 % of chronic patients (5 months to 9 years) improved
Ohta et al (1985)	CVD including infarction and hemorrhage	134	2ATA	Overall improvement in 72 % patients; CBF decreased with decrease in intracranial pressure; EEG and SSEP improved
Hao & Yu (1987)	Cerebral thrombosis (782) Cerebral embolism (16) TIA (31) Cerebral atherosclerosis	987	Pressure and duration?? 20-30 session	Improvement in 82.3 % of cases

Sugiyama et al (1987)	Cerebral infarction 1-3 months after onset	142	2 ATA for 75 min; 20 daily session	Very good results in 15% moderate improvement in 33%, and 20% showed no effect
Gusev et al (1990)	Acute stroke	220	1.2-1.3 ATA	Normalization of EEG, acid-base balance and decreased of raised free radicals by activation of lipid peroxidation

Appendix 3: Controlled Trails

Authors & Year	No. Of patients	Study Design	HBO Protocol	Results
(D. C. Anderson, et al., 1991)	39	Double-blind prospective study, patients treated within 2 w of onset	HBO 1 h at 1.5 ATA repeated every 8 h for total of 15 treatment	Same schedule of hyperbaric air in sham group. Study aborted as no dramatic improvement was noted in the HBO treated patient
(Nighoghossian, et al., 1995)	34	Randomized: half of the patients received HBO, the other half were treated in hyperbaric air, patient treated with 24 h of onset	HBO 40 m at 1.5 ATA, daily for 10 treatment	Control group received hyperbaric air. HBO was safe in these patients and there was an outcome trend favoring HBO therapy
(Rusyniak, et al., 2003)	33	Randomized, prospective, double-blind, sham-controlled study	HBO 1 h at 2.5 ATA (100% O2) o 1.14 ATA in the sham group	No differences between the groups at 24 h. At 3 months sham patient had a better outcome as defined by their stroke score.

Appendix 4: Random studies with HBOT treatment:

Author & year	No of patient/Animals	Study design	HBO protocol	Result
(Thurston, et al., 1973) Poor study	103 patients HBO Vs. 105 control group whom were treated conventionally in the same cardiac unit	Randomized control study following recent acute myocardial infarction	Patient were exposed for about 32 hours in the chamber only 57 of the 103 were in the chamber between 24-40 h the depth of treatment and the unknown	17 Patient whom were treated with HBO died (16.5%) compared with 24 (22.9 %), the overall mortality among the 80 patients who were exposed to a minimum of 4 hours in HBO was (11.3 %)
(Dennog, Radermacher, Barnett, & Speit, 1999) Fair study	7 Healthy volunteers (20-39 years) non-smokers	Experimental, to investigate whether intake of antioxidants may protect against HBO-induced DNA damage, we supplemented subjects with 3 volunteers with Vitamin E(800mg), and 4 with N-acetylcysteine (400mg) blood was taken before and after HBO sessions	2.5 ATA a total of 3X20 min periods interspersed 5 min of air breathing	HBO has no effect on "antioxidant power" of plasma in contrast, synthesis of the heat shock protein HSP70 which has been implicated to play an important role in cellular protection against oxidative stress which was found after a single treatment

Author & year	No of patient/Animals	Study design	HBO protocol	Result
(Speit, et al., 2000) fair study	14 healthy volunteer (non-smoker 24-30 year)	Experimental study, to indicate the DNA damage after HBOT, venous blood was taken before & after the session of HBO or a day after	100% O2 at 2.5ATA for 20 min interspersed with 5 min normal air breathing	Induction of DNA damage was found only after the first HBO exposure and not after further treatments. Further more, blood was taken 24h after HBOT was significantly protected against the induction of DNA damage by H2O2, indicating that adaptation occurred due to indication of antioxidant defenses.
(Dave, et al., 2003) Fair study	50 Wobbler mouse	Experimental were divided into 3 groups the animals were treated 28-30 days, the study is to find the mitochondrial dysfunction and delays onset of motor neuron diseases	First group (n=18) 2 ATA of 100% O2 for 1 h a day in a monoplace chamber, the last HBOT session was conducted 24 h before the mitochondrial isolation Control group (n=24) and not subjected to HBOT (n=8)	No significant differences were found in state 3 respiration rate of mitochondria isolated from the motor cortex of all 3 experimental

(Yasar, et al., 2003) Good study	45 Sprague-Dawley rat	Experimental study, to investigate the mechanism of HBO on oxidative stress in Acute Necrotizing Pancreatitis	Group I Sham group (n=15), Group II pancreatitis (n=15), Group III Pancreatitis group undergoing HBOT (n=15) for 5 days 2 sessions per day at 2.5 ATA for 90 min.	No complications related surgical method and HBO were detected. 4 rats on the second day and one rat on the third day died following pancreatitis induction in-group II. In Group III only two rats died on the third day of the study. (SOD) superoxide dismutase were lower in group II and III (MDA) malondiladehyde the level was higher in group II,III compare to I (GSH Px) Gluthathione peroxidase in-group II was lower compared to the other groups.
(Gulec, et al., 2004)	36 Sprague-Dawley rats	Experimental Acute Distal Colitis randomly divided into 3 groups	(Sham group I) colitis induced by acetic acid without any therapy, (Group I), colitis induced with HBOT (Group III) HBO was given for 5 days 2 sessions per day at 2.5 ATA for 90 min. (SOD), (MDA),(GSH Px) were measured in intestinal tissue	All rats developed diarrhea, (SOD) decreased significantly in-group II compare to group I, but increased in-group III. (MDA) were decreased in group III compare to group II (GSH Px) there was decreased in-group II and I compare to group III, which showed increase.
(W. Liu,	96 mice	Mice were randomly	HBO group, 60 min twice daily	Latency to seizures was significantly shortened in mice

et al., 2008)		assigned into 3 groups: HBO (n=32), HBA (n=32), NBA (n=32) The study was to investigate the mechanisms in increased sensitivity to convulsions & role of Nitric oxide after HBO	for 3 consecutive days (2.5 ATA 100% O2) 24 h after last exposure mice were expose to HBO (100 % 6 ATA) Hyperbaric air group, normal baric air group	after six HBO pre-exposure; the level of NO in hypothalamus in HBO group was increased. The number of NADPH-d positive cells and the levels of protein and mRNA of eNOS and nNOS in hypothalamus and hippocampus were increased. Conclusion= *after repeated HBO exposure, elevated NO may enhance the sensitivity to convulsions & this may lead to seizures during the subsequent oxygen exposure, prevention of seizures is needed when HBO is used as preconditioning methods.*
(Rubinstein, et al., 2009)	15 Male Sprague-Dawley rats,	Experimental study if HBOT improves GFR in rats with ischaemia/reperfusion renal injury: a possible role for the antioxidant. Oxidant balance in the ischaemic kidney	Rats with left kidney ischemia were randomly divided into 2 experimental groups, I (group + HBO, n=9) 90 min each 2 h, and 22 h after renal ischaemia. 100% O2 to 2.5 ATA	In the –HBO group, GFR was reduced by 94% compared with the untouched normal kidney (ischaemic: 0.06 ± 0.03 ml/min, normal: 1.02 ± 0.13 ml). In contrast, in the +HBO group, GFR of the ischemic kidney (0.36± 0.07 ml/min) was reduced only by 68% compared with the contra lateral normal kidney (1.12±0.12 ml/min) in line with these findings, HBO improved the vasodilateraly response to Ach as expressed in enhancement of both total and regional renal blood flow, in

			Group II (-HBO,n=6) was left at room atmosphere for 48 h	addition HBO reduced the formation of 4-HNE by 33% and 76% and increased SOD by 30% and 70% in the cortex and outer stripe region of medulla of the ischaemic kidney respectively. Conclusion= *HBO attenuates the decline in GFR following renal ischaemia, and improves endothelial-dependent vasorelaxation, suggesting that treatment with HBO may be beneficial in the setting of ischaemic ARF*