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THE INFLUENCE OF CHEMOPREVENTIVE COMPOUNDS ON FOOD CARCINOGEN ACTIVATION

Bachelor Thesis

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Declaration

I declare that this bachelor thesis was elaborated individually under the supervising of Doc. RNDr. Petr Hodek, CSc., and that all used references were cited properly.

Prague, 5th June 2006 .....

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Abbreviations

AFB_1	aflatoxin B_1
AFM_1	aflatoxin M_1
AhR	arylhydrocarbon receptor
AαC	2-amino-9H-pyrido[2,3-*b*]indole
BaP	benzo[a]pyren
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
BROD	benzyloxyresorufin *O*-dealkylase
COX	cyclooxygenase
CYP	cytochrome P450
DAS	diallyl sulphide
DASO	diallyl sulphoxide
$DASO_2$	diallyl sulphone
DiMeIQx	2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline
EPA	Environmental Protection Agency
EROD	ethoxyresorufin *O*-deethylase
FAD	flavinadenine dinucleotide
FMN	flavin mononucleotide
FMO	flavin-containing monooxygenase
G	glycyrrhizin
Glu-P-1	2-amino-6-methyldipyrido[1,2-a:3′,2′-*d*]imidazole
Glu-P-2	2-aminodipyrido[1,2-a:3′,2′-*d*]imidazole
GST	glutathione *S*-transferase
GST-P	placental form of glutathione *S*-transferase
HCA	heterocyclic amines
HIV	human immunodeficiency virus
HPV	human papillomaviruses
HTHQ	1-*O*-hexyl-2,3,5-trimethylhydroquinone
IARC	International Agency for Research on Cancer
IFP	2-amino-1,6-dimethylfuro[3,2-*b*]imidazo[4,5-*b*]pyridine
IQ	2-amino-3-methylimidazo[4,5-*f*]quinoline
IQx	2-amino-3-methylimidazo[4,5-*f*]quinoxaline

MeAαC	2-amino-3-methyl-9H-pyrido[2,3-*b*]indole
MeIQ	2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline
MeIQx	2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline
MFO	mixed function oxygenase system
MNNG	*N'*-methyl-*N'*-nitronitrosoguanidine
MROD	methoxyresorufin *O*-demethylase
NADH	nicotineamide adenine dinucleotide
NADPH	nicotineamide adenine dinucleotide phosphate
NBPA	*N*-nitrosobutylpropylamine
NDBA	*N*-nitrosodibutylamine
NDEA	*N*-nitrosodiethylamine
NDMA	*N*-nitrosodimethylamine
NDPA	*N*-nitrosodipropylamine
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NPYR	*N*-nitrosopyrrolidine
NSAIDs	nonsteroidal anti-inflammatory drugs
OSC	organosulphur compounds
PAH	polycyclic aromatic hydrocarbons
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
PPARα	peroxisome proliferator activated receptor α
PROD	pentoxyresorufin *O*-dealkylase
PXR	pregnane X receptor
t-BHQ	*tert*-butylhydroquinone
Trp-P-1	3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole
Trp-P-2	3-amino-1-methyl-5H-pyrido[4,3-*b*]indole
XREM	xenobiotic-responsive enhancer module

1 Introduction

Cancer belongs to one of the most widespread life-threatening diseases in world these days. Cancer is not only single disease, it represents a group of more than hundred diseases. All of these can be characterised by the uncontrolled growth of an abnormal cell producing a population of cells that have acquired the ability to multiply and invade surrounding and distant tissues.

Carcinogenesis is a multi-step process through which cancer develops. This process begins when a carcinogen enters a body (except endogenous compounds). Carcinogens can be classified as chemical, physical or biological factors. Chemical compounds that can cause cancer are called chemical carcinogens. They act directly (direct carcinogens) or only after activation (non-direct carcinogens, also called procarcinogens). Procarcinogens can be activated either by enzymes that are regularly present in organism metabolising endogenous compounds or by micro flora in the body.

If the carcinogen is direct acting, the only way how to decrease the incidence of cancer is by decreasing the exposure to this compound. On the other hand, if the activation is necessary there is another way in inhibition and induction of enzymes involved in activation and detoxification process, respectively.

The idea of cancer prevention is accepted since 1950's and the term "chemoprevention" was introduced by Michael Sporn in 1970's. Recently, many researchers are trying to develop chemical compounds which could provide chemopreventive effects on organism. Many of these agents naturally occur in plants (e.g. broccoli, green tea, curcuma, etc.).

Preparations, containing for example natural and synthetic flavonoids, vitamins, fibre, fruit and vegetable extracts are produced today in a large scale. But these mixtures affect many enzymes in organism and there is a question if the preparation provides only beneficial effect on organism. However, it is possible that they can also act negatively and even enhance carcinogenic potential of other compounds. It was observed that some compounds that do not show mutagenic activity by itself can enhance mutagenic activity of other compounds. Mechanism of this process consists in modulation of enzymes involved in metabolism of this compound. The detailed insight into the process of chemoprevention is presented in the following review.

2 Aim of the work

This work is focused on the modulation of cytochromes P450 (phase I enzymes, involved in activation of carcinogens) by chemopreventive compounds with the special attention to their possibly negative interactions that may lead to enhanced risk of cancer development. The specific aim of the review is to:

1. find particular cytochrome P450 isoforms that are inducible by chemopreventive compounds and are involved in activation of carcinogens, and
2. asses interactions between carcinogen and chemopreventive compounds metabolism, under exposure to these compounds (sequential or/and simultaneous).

3 Metabolism of xenobiotics

The metabolism of xenobiotics is a multi-step process catalyzed by variety of enzymes. In general, this process can be divided in two phases.

In the first, derivatization, phase (phase I) a xenobiotic is metabolized into more polar compound to be excreted from the organism more easily and/or to be accepted by enzymes of the following second phase. These reactions are, basically, oxidation, reduction and hydrolysis.

The metabolite, resulting from phase I, is conjugated in the second phase (phase II) with endogenous polar compound leading to more water soluble compound, making it easier to be excreted. This process is often followed by transport of this metabolite out of the cell, sometimes incorrectly called phase III.

3.1 Phase I

Phase I reactions include microsomal monooxygenations, cytosolic and mitochondrial oxidations, co-oxidations in the prostaglandin synthetase reaction, reductions, hydrolyses, and epoxide hydrations [1]. These reactions expose or introduce more polar group (e.g. OH, NH_2, SH, COOH) into molecule resulting in only a small increase in hydrophilicity. However, these polar groups can be conjugated with other compounds in the second phase.

Enzymes involved in this phase are for example cytochrome P450-dependent monooxygenase system, flavin-containing monooxygenases, prostaglandin synthetase, alcohol and aldehyde dehydrogenases, esterases, amidases and epoxide hydrolyses.

The key role in phase I reactions play cytochrome P450-dependent monooxygenase system (CYP) and flavin-containing monooxygenases (FMO), that are mainly localized in endoplasmic reticulum. They both are NADPH or NADH dependent enzymes. The overall reaction of these enzymes is:

$$RH + O_2 + NAD(P)H + H^+ \rightarrow NAD(P)^+ + ROH + H_2O.$$

CYPs are one of the most studied enzymes of this phase because they play a major role in activation reactions.

3.2 Phase II

Products of the first phase usually containing hydroxyl, amino, carboxyl or epoxide group or halogen are conjugated with endogenous compounds such as sugars, amino acids,

glutathione, sulphate or phosphate. These reactions are catalyzed by phase II enzymes and usually result in less toxic, more polar and more readily excreted compounds than the parent ones.

Enzymes involved in this phase are for example glutathione *S*-transferase, sulfotransferase, methyltransferase, glucuronyltransferase or acyltranferase.

3.3 Activation reactions / enzymes

Although xenobiotics are usually detoxified during this metabolizing process, some reactions produce reactive metabolites or compounds which are even more toxic than the parent compound. This process is usually called metabolic activation or bioactivation. The relationship between metabolism and toxicity of chemicals is described in Figure 3-1.

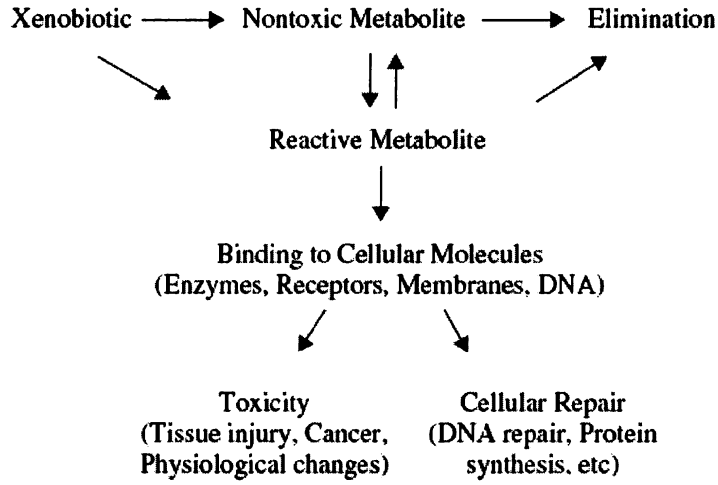


Figure 3-1 The relationship between metabolism (activation, detoxification) and toxicity of a chemical (adapted from [1])

Reactive metabolites are most frequently electrophilic compounds and radical molecules. Electrophilic compounds have positively charged center that can easily react with nucleophilic parts of molecules, such as nucleic acids and proteins. Radicals act directly (also called free radicals) or form other radicals and act as radical generators. These radicals can interact with oxygen to produce reactive oxygen species which might damage cell membranes, DNA, etc. Reactive metabolites can be detoxified by phase II enzymes.

Xenobiotics are usually transformed by more than one pathway and by more than one enzyme into many different compounds. The majority of these products are less toxic but the minority of formed reactive compounds are of a great importance. The activation

reaction may become more dominant in situations when nutrient or chemical compound positively modulate the particular enzyme activity.

Table 3-1 Important enzymes involved in activation reactions

Type of Reaction	Enzyme
Oxidation	Cytochrome P450s
	Prostaglandin synthetase
	Flavin-containing monooxygenases
	Alcohol and aldehyde dehydrogenases
Reduction	Reductases
	Cytochromes P450
	Gut microflora
Conjugation	Glutathione transferases
	Sulfotransferases
	Glucuronidases
Deconjugation	Cysteine *S*-conjugate β-lyase
Hydrolysis	Gut microflora, hydrolyses

Adapted from [1]

Almost all enzymes which are involved in metabolism of xenobiotics can form reactive intermediates (Table 3-1). The majority of reactive products are produced by oxidation reactions. The predominant pathway in activation oxidation reactions is catalyzed by cytochrome P450 monooxygenase system. Phase II enzymes are not as important as phase I enzymes in the activation processes. They are mainly detoxifying and only a small number of activation reactions is known.

3.4 Modulation of xenobiotic metabolism

The metabolism of exogenous compounds can be affected by various conditions, e.g. medicinal drugs, environmental compounds, nutrition. This influence can result in induction and/or inhibition of enzymes and can lead, for example, to enhanced activity of enzymes involved in detoxification reactions (positive effect) but also to enhanced activity of biotransformation enzymes leading to carcinogen activation (negative effect).

In mammals, the birth is the limiting step that initiates an increase of hepatic enzyme activity, including phase I and phase II xenobiotic metabolizing enzymes (CYP, reductase, glucuronosyltransferase, glutathione transferase, etc.). Time needed to reach normal level of these enzymes is dependent on type of enzyme (e.g. monooxygenase in rats 30 days, glutathione transferase in rats 140 days) and animal species. Levels of these enzymes are changing through whole life, usually decreasing with age [1].

There are extensive differences marked between genders starting to occur at puberty. Differences in humans are not such as high as in other species. In humans monooxygenases (CYP) activity is frequently under control of sex hormones.

3.4.1 Nutritional effects

Metabolism of xenobiotics involves many enzymes and each of them has different requirements for cofactor, prosthetic group or endogenous co-substrates. Because of this there are many nutrients that affect activity of these enzymes and metabolism of xenobiotics.

Phase I enzymes: The nutrients important for CYP activity are B complex vitamins (niacin and riboflavin which are involved in formation of NADPH, FAD, FMN), essential amino acids, iron (essential micronutrient), for heme synthesis are also needed pantothenic acid (synthesis of coenzyme A), pyridoxine and copper (ferroxidase system involved in incorporation of iron to porfyrin structure).

Phase II enzymes: Nutrients important for these enzymes are many different vitamins, minerals, amino acids and many other compounds. For example formation of mercapturic acid requires essential amino acids, pantothenic acid (coenzyme A synthesis) and phosphorus (needed for ATP synthesis).

Nutritional constituents are very important in modulation of metabolism. Low dietary protein levels, in general, inhibit liver monooxygenases. In closer look there are differences among CYP isoforms, e.g. there is almost no effect of low-protein diet on CYP2E1 in rats [1]. High-carbohydrates diet has almost the same effect as low-protein in rats. It has been also demonstrated in humans that changing ratio of protein to carbohydrates modulates enzyme activity, while changing ratio of fat to protein has no effect. Level of lipids has also many effects on metabolism, e.g. deficiency of unsaturated fatty acids (fats) leads to decreased level of CYP [1]. Starvation has similar effect as low-protein diet on animals (monooxygenase activity is decreased).

Micronutrients, such as vitamins, have different effects on CYP isoforms. In most cases, deficiency of vitamins decreases levels of CYP and inhibits monooxygenase activity. Mineral micronutrients have almost no significant effects but for example calcium and magnesium deficiency decreases monooxygenase activity and deficiency of iron increases its activity (but not accompanied by increased level of CYP) in immature rats [1].

3.4.2 Chemical effects

The study of the metabolism and toxicity of xenobiotics *in vitro* is usually focused on a single compound. However, *in vivo* there is different situation when many compounds are influencing one enzyme at the same time. In general, enzymes are exposed to more xenobiotics or compounds simultaneously and every single compound has its own metabolic pathway.

Enzymes can be divided in two groups, inducible and constitutive enzymes. Inducible enzymes, such as CYPs, can be induced by inducers while level of strictly constitutive enzymes in organism cannot be increased by any compound. Some enzymes are both inducible and constitutive (are constitutively expressed and their level can be increased by other compounds).

Metabolism of a xenobiotic can be influenced by the presence of another compound not only when the enzyme involved is inducible. Four basic situations might occur under simplified conditions involving only two compounds, A and B:

- When compound A, toxic only after activation, is administrated in presence of an inhibitor of its metabolism (activating enzyme), the toxic effect will be reduced.
- When compound A, toxic only after activation, is administrated after exposure to an inducer of the activating enzyme, there would be increase in toxic effect.
- Compound B, a toxicant, metabolically detoxified, given in the presence of inhibitor of the detoxifying enzyme, would appear more toxic.
- When compound B, a toxicant, metabolically detoxified, is administrated after exposure to an inducer of the detoxifying enzyme, there would be decrease in toxicity.

Many xenobiotics that are initially enzyme inhibitors can ultimately become inducers. Inhibition of enzymes is observable in significantly shorter time than enzyme induction. This effect can be explained by induction and inhibition mechanism. Inhibitors are directly interacting with the enzyme, where inductors are usually stimulating signaling pathways leading to synthesis of the enzyme. Therefore, one dose of such a compound can initially decrease the activity of enzyme, e.g. CYP, due to inhibition and then can act as an inductor of this and/or other enzymes.

Cytochromes P450 are inducible enzymes involved in xenobiotic metabolism of the highest importance. Moreover, other enzymes involved in xenobiotic transformation, such as FMOs, glutathione *S*-transferase, epoxide hydrolase and UDP glucuronyltransferase, are inducible too.

Important effects observed are synergism and potentiation. Both, in general, result in increase of toxicity when two compounds are given simultaneously or sequentially than would be expected from a consideration of the toxicities of these compounds given alone. Term "synergism" is usually used when one of these compounds alone has no or only little intristic toxicity and term "potentiation" is used when both compounds have intristic toxicity.

Antagonism means the opposite of synergism and potentiation. This term is defined in situations when toxicity of two or more compounds administered together or sequentially is lower than would be expected from a consideration of their toxicities when administered individually.

3.5 Cytochrome P450 (EC 1.14.14.1)

Cytochrome P450 is one of the most important enzymes involved in biotransformation of xenobiotics. This enzyme together with NADPH: CYP oxidoreductase and membrane phospholipids (e.g. phosphatidylcholin), and possibly with cytochrome b_5 and NADH: cytochrome b_5 oxidoreductase (e.g. human CYP3A4, CYP3A5 and CYP2E1 [1]), compose mixed function oxygenase system (MFO).

The first experimental evidence relating to cytochromes P450 was discovered in 1955 by Axelrod and Brodie. They identified an enzyme system in the endoplasmic reticulum of the liver which was able to oxidize xenobiotic compounds. In 1958 William and Klingenberg detected carbon monoxide binding pigment in liver microsomes. It was named P450 because its reduced form, in complex with carbon monoxide, shows strong absorption band at 450nm.

Cytochrome P450s manage activation of molecular oxygen and binding of one atom of oxygen to substrate molecule. The second atom of oxygen is reduced to water. As the donor of electrons NADPH: cytochrome P450 reductase or NADH: cytochrome b_5 reductase is involved.

Cytochrome P450s were found in a wide variety of living organisms including microorganisms (yeast, bacteria), animals, plants and fungi. Soluble cytosolic forms were

found in bacteria and yeast. In eukaryotes, cytochromes P450 are transmembrane, localised in inner mitochondrial membrane, smooth endoplasmic reticulum membrane, and in lower amounts also in rough endoplasmic reticulum membrane and nuclear membrane, and because of this they are insoluble.

Until these days more than 2000 CYPs have been found. They categorized into families and subfamilies according to their sequence similarities, new nomenclature of CYPs (proposed firstly in 1987, lastly changed in 1996). Isoforms that have more than 40% of sequence identity are classified in one group which is symbolized by the first number. Members of subfamily have more than 55% of sequence identity in mammals and more then 46% in non-mammals, and are marked with the capital letter. The particular isoform of cytochrome P450 is marked with another number. For example: CYP2E1, where 2 is family number, E is subfamily letter and 1 exact isoform of cytochrome. Term "isoform" means proteins which are coded by distinct genes. However, sequences with less than 3% divergence are thought to be allelic variants of one isoform.

3.5.1 Structure

Cytochrome P450 contains porfyrin structure, heme-ring (non-protein part of enzyme), and apoprotein part (protein part). Heme-ring contains a central iron atom which is able to provide six valences for electron donation ligands. Four of the valences are engaged in binding of nitrogen atoms from porfyrin ring structure, ferroprotoporfyrin IX. The fifth valence is occupied by thiolate anion (negatively charged sulphur atom) from cysteine of apoprotein. The last, sixth ligand, of iron atom is usually an oxygen atom from water molecule, hydroxyl or carboxyl group from apoprotein or from some exogenous compounds.

The apoprotein part of cytochrome consists of between 490 to 520 amino acids, depending on the particular isoform. The protein is made of at least three functional domains. The first domain binds heme, the second binds reductase (enzyme co-operating with CYP) and third binds substrate. The active site of protein consists of heme and substrate binding domains. Sequence of this heme binding part is highly conserved while other parts of cytochrome sequence are rather variable except of key amino acid residues which are necessary for reductase binding. The common feature of substrate binding sequence is a high content of hydrophobic amino acids.

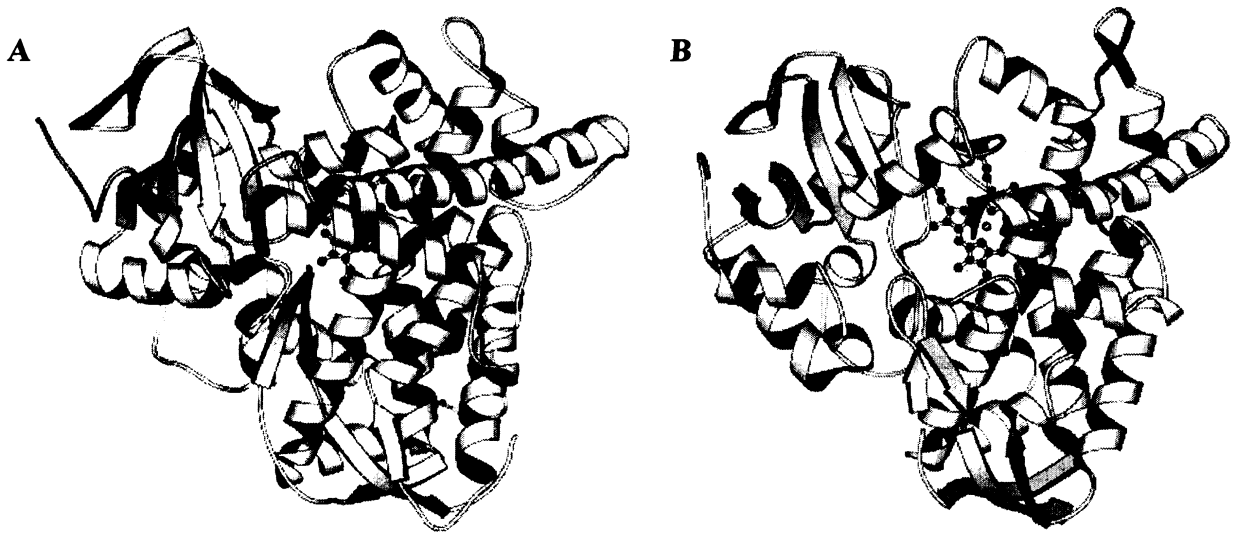


Figure 3-2 3D structure of cytochromes: A, P450 BM-3 (CYP102) from *Bacillus megaterium* and B, P450terp (CYP108) from *Pseudomonas sp.*

Individual cytochromes P450 isoforms usually differ in their relative molecular weight depending on number of amino acids in sequence. Relative molecular weight of mammalian cytochromes is spanning in the interval of 46 - 60 thousands. The 3D structure of cytochrome (crystallized cytosolic soluble CYP from prokaryotes) and the orientation of cytochrome P450 system in the membrane is shown in Figure 3-2 and Figure 3-3.

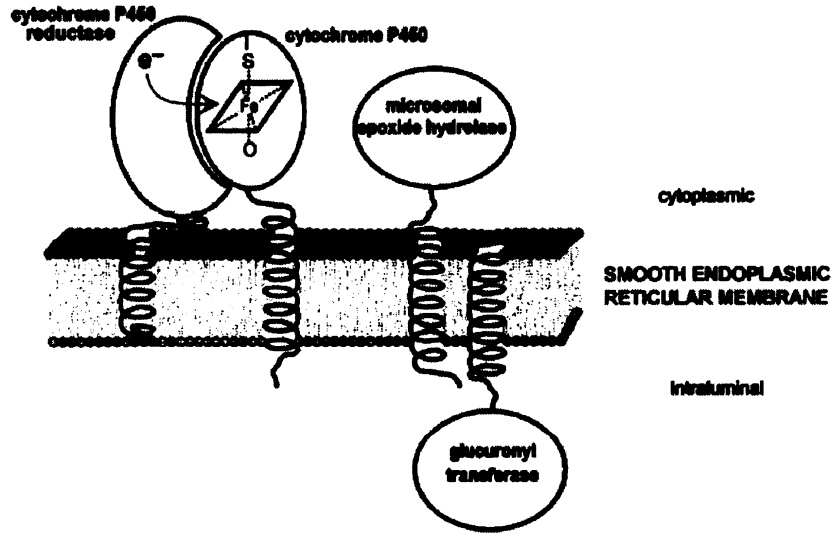


Figure 3-3 Schematic representation of the orientation of cytochrome P450 and its reductase, microsomal epoxide hydrolase and glucuronyl transferase in the smooth endoplasmic reticulum membrane (adapted from [7])

3.5.2 Function

Cytochromes P450 provide various functions depending on the isoform. For example in human, there are two groups of cytochromes, first metabolising sterols, fatty acids, eicosanoids, vitamins and other endogenous compounds and second mainly

catalyzing transformation of xenobiotics. The function of all cytochrome P450 isoforms has not been discovered yet.

The general catalytic cycle of monooxygenase reaction of cytochrome P450 proceeds through many steps in cooperation with another two enzymes associated (Figure 3-4), mainly NADPH: cytochrome P450 reductase and in minor extend NADH: cytochrome b_5 reductase. These are flavoprotein enzymes, NADPH: cytochrome P450 reductase contains one mole of each flavinadenin dinucleotide (FAD) and flavin mononucleotide (FMN). Other compounds involved in this cycle are phospholipids, mainly phosphatidylcholin which seems to be involved in coupling of the reductase to cytochrome and in the binding of substrate to cytochrome P450 [1].

First step in catalytic cycle of monooxygenase reaction of CYP is substrate binding to oxidized CYP (Fe^{3+}). Oxidized, substrate free form of CYP is hexacoordinated (low spin state) and the sixth ligand of iron is usually an oxygen atom from water or from amino acid from side chains of CYP sequence. When substrate reaches the active site of enzyme then the sixth ligand is expulsed resulting in conformational changes of cytochrome structure and the iron atom is shifted to heptacoordinated form (high spin state).

The second step is one electron reduction catalyzed by NADPH: cytochrome P450 reductase by which reduced CYP (Fe^{2+})-substrate complex is formed. This form of enzyme (pentacoordinated and reduced) can bind another ligands, such as molecular oxygen or carbon monoxide.

Interaction with molecular oxygen forms ternary oxygenated complex (hexacoordinated) that can accept the second electron. Several products (complexes) are formed in this reaction. One of them is peroxide anion derivative of the substrate-bound hemoprotein. Under some conditions hydrogen peroxide (cytotoxic for cells) is released and oxidized CYP complex is formed, this reaction is called uncoupling. Regularly dioxygen molecule is split and one oxygen atom is bound to CYP-substrate complex forming complex where one atom of oxygen is bound to iron in heme, and in another step oxygen radical is formed. This radical is highly reactive and can pull out one electron from appropriate substrates to form hydroxyl radical and substrate radical. These radicals can recombine to form hydroxy-derivate of substrate and the oxidized (original) form of CYP (Fe^{3+}, hexacoordinated). The second atom of oxygen is reduced by two protons into water molecule.

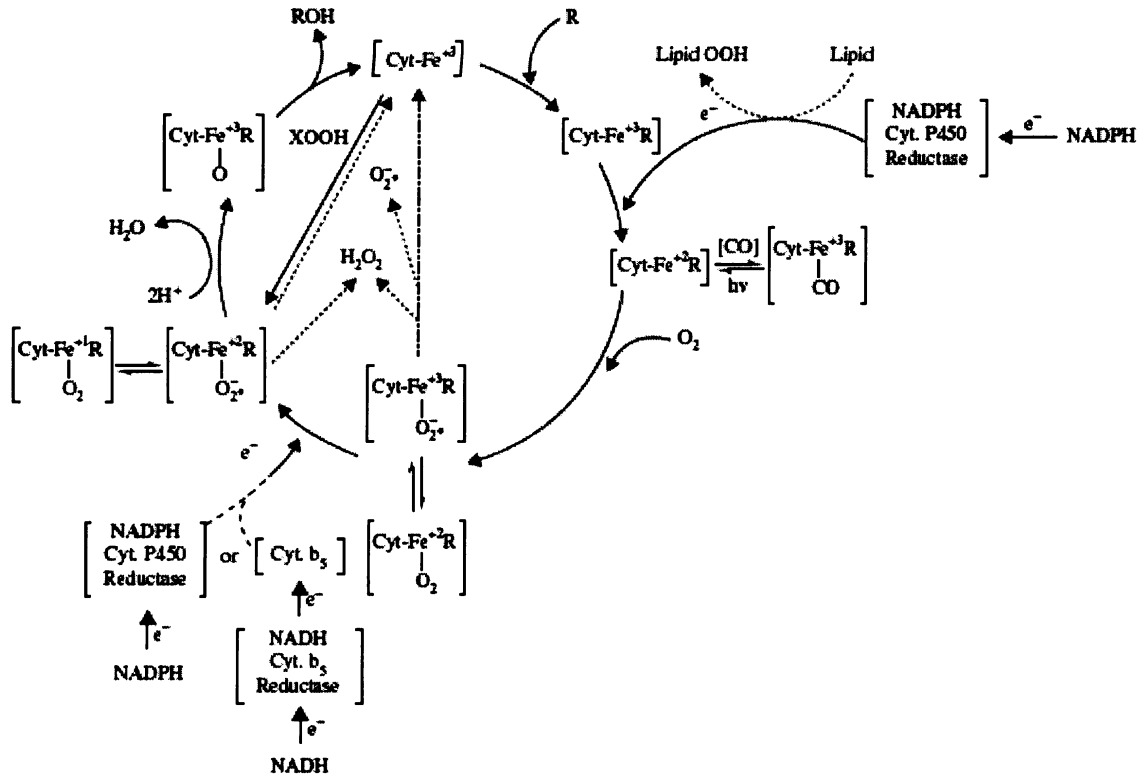


Figure 3-4 **Generalized scheme of CYP monooxygenase function (adapted from [1])**

3.5.3 Modulation of activity

There are many factors influencing activity of cytochromes P450 that can lead to inhibition and/or induction of these enzymes. Both, inhibition and induction may cause decrease or increase of xenobiotic toxicity. These factors are for example nutrition, physiological, chemical and environmental effects (discussed above in chapter 3.4).

Cytochrome P450 activity

Activity of particular cytochrome P450 is usually measured as the rate of specific substrate conversion. Most widely used substrates are *O*-alkoxyresorufins which are dealkylated by CYPs (Table 3-2). Methoxyresorufin is highly specific for CYP1A2, ethoxyresorufin is specific for CYP1A1, pentoxyresorufin is specific for CYP2B1/2 and benzyloxyresorufin is metabolized by CYP1A1/2, CYP2B1/2 and CYP3A.

Table 3-2 **Specific methods and substrates used to determine CYP activity**

Method, reaction		Specific CYPs
benzyloxyresorufin *O*-dealkylase	BROD	1A1/2, 2B1/2, 3A
methoxyresorufin *O*-demethylase	MROD	1A2
ethoxyresorufin *O*-deethylase	EROD	1A1
pentoxyresorufin *O*-dealkylase	PROD	2B1/2

Inhibition

Enzyme inhibition leads to decreased activity of this enzyme. Inhibition can be either reversible or irreversible (e.g. suicidal substrate is metabolized by this enzyme to reactive metabolite that damages the enzyme). Inhibition of CYP can be either reversible or irreversible involving many mechanisms.

Reversible inhibition involves no covalent binding and is usually classified into competitive, uncompetitive and non-competitive inhibition. These types are not rigidly separated and many intermediate cases exist. Process when two substrates are competing for the same active site of the enzyme is called competitive inhibition. For CYPs are expected inhibitors, type I ligands, that are binding in active site but not to heme iron. If inhibitor cannot react with free enzyme but can react only with complex substrate-enzyme to cause decrease of enzyme activity, the inhibition is called uncompetitive. Non-competitive inhibitors can bind either to enzyme or to complex enzyme-substrate and affect rate of activity of enzyme (positively or negatively).

Irreversible inhibition involves in most cases formation of covalent or other stable bond, or disruption of the enzyme structure. This process is irreversible and enzyme cannot be regenerated.

Induction

CYP induction is based on various mechanisms as increased transcription of DNA, increased translation of mRNA to protein, mRNA and protein (CYP) stabilization. According to this, the increase of activity is correlating with enhanced synthesis of the enzyme, not with the activation of enzyme already synthesized.

On the **level of transcription**, there are involved four major pathways, and inducers are classified in few groups that are differing in mechanism. These groups are: phenobarbital-type inducers, dexamethasone/rifampicin-type inducers, TCDD-type inducers and other inducers, such as ethanol.

Major classes of cytochrome genes are selectively regulated by ligand-activated nuclear receptors, which are responsible for transforming of signal into cellular responses. Nuclear receptors contain ligand-dependent transactivation domain (N-terminal), DNA-binding domain (containing two zinc finger motifs) and ligand binding domain (C-terminal). In most cases the ligand-dependent hetero or homo-dimerization of receptors (usually with retinoid X receptor, RXR) is required [3].

Eukaryotic DNA transcription is regulated also on the level of chromatin structure. In general, chromatin has two major forms, euchromatin (condensed) and heterochromatin (more relaxed). Transcription can proceed only when chromatin is not so condensed and DNA is accessible for transcriptional enzymes. DNA is wrapped around histone proteins, and acetylation of histones is one of two basic regulation mechanisms. Another one is methylation of cytosine in DNA [3, 5].

After ligand is bound to nuclear receptor, conformational change usually occurs in ligand binding site so that other molecules can bind there. These molecules are usually co-activators or co-repressors. Co-activator activates transcription by histone acetylation (mediated by acetyltransferase) while co-repressor binding results in histone deacetylation and decreased transcription [5].

Induction is relatively slow process in comparison to inhibition. It usually takes hours until an increase of CYP activity is apparent. This time depends on the inducer compound and induction mechanism involved. For example induction by rifampicin, inductor of CYP3A4, 1A2 and 2C, is apparent within 24 hours but by phenobarbital it requires almost a week to reach the maximal induction [5].

Highly inducible human cytochromes P450 involved in metabolism of xenobiotics are members CYP1A, CYP2B, CYP2C, CYP3A, and CYP4A subfamilies [6]. Human inducible isoforms involved in drug (xenobiotic) metabolism are mainly CYP1A2, 2C9, 2C19, 2E1 and 3A4 [5].

Aromatic hydrocarbon receptor

Aromatic hydrocarbon receptor (AhR) is cytosolic protein that can bind compounds such as TDCC, 3-methylchloranthrene and polycyclic aromatic hydrocarbons resulting in complex formation. Then AhR-ligand complex is translocated to the nucleus and forms a dimer with another protein known as AhR nuclear translocator (ARNT). This complex interacts with specific sequences of DNA (xenobiotic responsive elements, XREs) in the nucleus. Effect resulting from this interaction is thought to be in bending of the DNA which increases level of transcription and also increases level of proteosynthesis.

This particular receptor is responsible for regulation of CYP1A and CYP1B expression [2].

Phenobarbital-type induction

In this type of induction, there are many elements and factors cooperating including nuclear factor 1 (NF1) and nuclear receptor binding sites 1 and 2 (NR1, NR2) resulting in increased DNA transcription. On DNA there is sequence called phenobarbital-responsive enhancer module (PBREM) which interacts with constitutive active receptor (CAR). Then forming heterodimer with the retinoid X receptor (RXR), CAR reacts with PBRE NR sites. CAR and RXR are constitutively expressed receptors and their heterodimer is in inactive form binding two endogenous androstane steroids. Displacement of these molecules leads to increased activity of PBRE related genes.

Cytochrome subfamilies under this type of expression control are for example CYP1A, CYP2B, CYP2C and CYP3A [2].

Pregnane X receptor

There is the region on DNA called xenobiotic-responsive enhancer module (XREM) consisting of two nuclear receptor binding sites (dNR1 and dNR2) and another one important nuclear receptor site (dNR3). The latter is thought to be involved in activation of gene expression and is distant from XREM several hundred base pairs downstream. Pregnane X receptor (PXR) is nuclear receptor involved in CYP3A induction which binds to promoter regions and activates its expression.

Peroxisome proliferators

Peroxisome proliferator-activated receptor α (PPARα) forms heterodimer with RXR and binds to DNA as a response to peroxisome proliferating chemicals. Result of this process is in activation of DNA transcription. This regulation mechanism is for example responsible for CYP4A induction and also for other enzymes involved in β-oxidation of fatty acids regulation [2].

Other induction types

Regulation of CYP2E1 expression is not primarily on the level of transcription. Induction of CYP2E1 is not usually accompanied by high levels of mRNA and regulation is thought to be post-transcriptional. Mechanisms involved are stabilization of existing proteins (inhibition of ubiquitin-mediated proteolysis). Sometimes level of mRNA is increased due to stabilization of mRNA. Because of this, induction of CYP2E1 cannot be determined directly by PCR techniques.

CYP3A1 is also partly regulated by stabilization of mRNA and proteins.

3.5.4 Human cytochromes P450

There are extensive differences among cytochromes P450 in species. This chapter is focused on human CYPs. To date, 57 cytochromes P450 included in 18 CYP families have been identified in human genome. Of the 57, most have been examined appear to be expressed primarily in the endoplasmic reticulum and only six are located exclusively in mitochondria [2]. In human, CYPs are mainly expressed in liver, lungs, gastrointestinal tract (mainly small intestine) and kidney [1].

CYPs have many different catalytic activities. From these whose activities are known (42 CYPs) are 14 involved in steroidogenesis, 4 in metabolism of vitamins (A and D), 5 in metabolism of eicosanoids, 4 in metabolism of fatty acids and 15 in xenobiotic metabolism [2]. However, one particular CYP isoform usually catalyzes more than one reaction, for example xenobiotic metabolising cytochromes are also involved in metabolism of fatty acids and steroids (oxidation). Classification of human CYPs based on major substrate types is shown in Table 3-3.

Xenobiotic metabolising enzymes are mainly of CYP families 1, 2 and 3. Cytochromes involved in steroid metabolism are usually invariable among individuals in contrast to xenobiotic metabolising enzymes which vary considerably [2]. Cytochromes involved in drug metabolism are mainly CYP3A4 (and 3A5), 2D6 and 2C9 making together about 75% of drug metabolism. Other important isoforms involved are CYP1A2, 2C19 and 2E1. All these enzymes are responsible for 90-95% of drug metabolism [2].

Major enzymes involved in metabolism of chemical carcinogens are CYP1A1, 1A2, 1B1, 2A6, 2C9, 2E1, 3A4 [2].

Table 3-3 Classification of Human cytochromes P450 based on major substrate class (adapted and modified from [2])

Sterols	Xenobiotics	Fatty acids	Eicosanoids	Vitamins	Unknown
1B1	1A1	2J2	2F2	24	2A7
7A1	1A2	4A11	2F3	26A1	2R1
7B1	2A6	4B1	2F8	26B1	1S1
8B1	2A13	4F12	5A1	27B1	2U1
11A1	2B6		8A1		2W1
11B1	2C8				3A43
11B2	2C9				4A22
17	2C18				4F11
19	2C19				4F22
21A2	2D6				4V2
27A1	2E1				4X1
39	2F1				4Z1
46	3A4				20
51	3A5				26C1
	3A7				27C1

CYP1 family

Three known human members of this group are CYP1A1, 1A2 and 1B1. This family primarily prefers highly planar structures, e.g. CYP1A1 neutral planar polycyclic aromatic hydrocarbons (PAHs), CYP1A2 polyaromatic and heterocyclic amines and amides. Because of this CYP1A subfamily is associated with metabolic activation of many procarcinogens and mutagens including benzo(a)pyren, aflatoxin B1, dimethylbenzantracen, β-naphtylamine, 4-aminobiphenyl, 2-acetylaminofluorene and benzidine [1]. Typical reaction of this subfamily is formation of epoxide and epoxide diols.

All these isoforms of cytochrome P450 are inducible and can be induced by for example planar PAH compounds which are also metabolized by these enzymes. CYP1A2 is even constitutive.

CYP1A1 is expressed in lung, several extrahepatic tissues (kidney, gastrointestinal tract, placenta, skin [8]), peripheral blood cells [2] and after induction also in liver (not expressed constitutively) [1]. CYP1A2 is constitutively expressed in liver [1, 4, 8] and CYP1B1 is expressed in liver [8] and many extrahepatic tissues including lung, kidney [2, 8], skin, prostate, uterus and fetus [8].

CYP2 family

This family contains ten subfamilies, from those five were found in mammalian liver. The most important members of this family in humans are CYP2A6, 2B6, 2C8, 2C9, 2C19, 2D6 and 2E1.

CYP2A6 is inducible [8] and is primarily expressed in liver [1, 8] but is also found in lung [2, 8] and several extrahepatic tissues [2]. This isoform is responsible for metabolism of nicotine (up to 80%) and also for activation of some procarcinogens, e.g. aflatoxin B1, 1,3-butadiene, 2,6-dichlorobenzonitrile and nitrosamines (NNK) [1, 8].

CYP2B6 is constitutive and inducible [8] and is expressed in liver [1, 2, 4, 8] (constitutively expressed in small amount in almost all liver samples), lung [2, 8] and gastrointestinal tract [8]. This isoform plays role in activation of organophosphates and chlorpyrifos (pesticide) and is involved in metabolism of many clinical drugs (e.g. cyclophosphamide, barbiturates) [1].

CYP2C subfamily contains only constitutive isoforms which are expressed in pharynx, gastrointestinal tract, lung [8] and liver [1, 2, 4, 8]. Members of this family in humans are CYP2C8, 2C9, 2C18 and 2C19. This subfamily is involved in several drug

metabolisms (e.g. warfarin, diclofenac, diazepam, verapamil, omeprazol). CYP2C8 and 2C9 also activate benzo(a)pyren, but they play a minor role.

CYP2D6 is constitutively expressed in gastrointestinal tract [8], brain and liver [2, 4, 8], and is involved in metabolism of many drugs (e.g. debrisoquine, propafenone, codeine, tramadol, tolterodin).

CYP2E1 is constitutive and inducible [8] and is expressed in liver [2, 4, 8], lung [2, 8], placenta [8] and other extrahepatic issues [2, 4, 8]. This enzyme is involved in metabolism of some drugs (e.g. paracetamol)

CYP3 family

Two major isoforms of this family are found in human, CYP3A4 and CYP3A5. Both are constitutive and inducible [8] and are involved in metabolism of many important drugs (e.g. nifedipine, midazolam, erythromycin, cyclosporin). More important (high expression) of these is CYP3A4 which is highly expressed in liver. Other tissues that contain CYP3A are small intestine (3A4) [2], lung [2, 8], uterus, fetus, gastrointestinal tract and placenta [8].

These enzymes are also responsible for activation of some procarcinogens and mutagens including aflatoxin B1 and G1, polycyclic hydrocarbons and polycyclic hydrocarbon-derived dihydrodiols [1, 2, 80].

4 Carcinogenesis

Process of carcinogenesis can be divided in three phases: initiation, promotion and progression (promotion phase can be omitted in some cases).

Initiation is the first phase of carcinogenesis, in which non-direct acting carcinogen is activated. Initiation factors (carcinogens) interact with DNA and cause changes in DNA (e.g. alkylation of DNA, forming of covalent adducts, single-strand and double-strand breaks, DNA-DNA cross-linking, incorporation of viral DNA in host DNA, intercalations to DNA, forming of base dimers). When these changes are misrepaired by DNA-repair system or not recognized by the cell, then the outcome can be aberrant transcription of the affected DNA, altered expression or no effect if the damage is located within non-coding or non-regulatory DNA [3]. Critical gene targets are coding regulation proteins of cell cycle and are called proto/oncogenes and tumour suppressor genes.

Initiated cell is the cell with misrepaired mutation of DNA that activates oncogenes or deactivates tumour suppressor genes. For the beginning only this one cell is directly affected but when this cell divides then the mutation is inherited and fixed in DNA of daughter cells.

Mutations of DNA occur regularly in organism. Every day there are about $10^6 - 10^4$ DNA mutations in organism [9] and if the organism has regularly efficient DNA-repair system than all of these mutations are repaired.

When the initiated cell is not destroyed by immune system than the cell is waiting for another signal (factor) to proceed to **promotion** phase. Promotion factors are also causing DNA damages but usually non-directly by changing the cell conditions that result in dividing of the cell with interrupted regulation of differentiation and cell communication. These cells proliferation is partly controlled, and they divide forming benign tumour. Promotion factors have concentration thresholds and when this concentration is reached, their effect is dose-dependent until reaching maximum response level (saturation of physiological targets) [3].

Progression phase involves further genetic damage (agent-mediated or spontaneous). Transformation to this phase involves progression factors which are changing cell to non-regulated and undergo malignant tumour conversion.

Some cells of the tumour can be transported by blood or lymphatic system to other tissues or organs in organism forming secondary tumours, so called metastases. This whole process is extremely slow taking even tens of years including lag (latent) phase when the

cell is waiting for another signal or factor to occur. The scheme of whole carcinogenesis is shown in Figure 4-1.

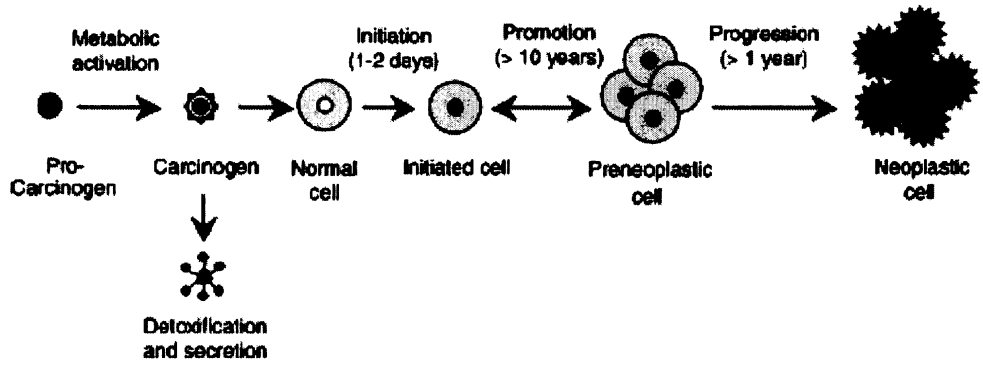


Figure 4-1 Scheme of multistep carcinogenesis (adapted and modified from [36])

In general, cells can be classified as normally proliferative and generally quiescent (need some proliferative stimulus to divide). In normally proliferative cells there is greater chance of neoplastic development (promotion phase can be omitted).

The most studied phase is initiation of carcinogenesis because it is the first and critical step in the whole process. Within this stage there could be a possibility how to decrease the incidence of cancer if the mechanism of cancer initiation was known.

5 Carcinogens

Carcinogens are usually classified in two groups: DNA-damaging agents and epigenetic (nongenotoxic) agents. First mentioned are mutagenic in *in vitro* assays, while the latter are not mutagenic.

Epigenetic agents are considered to alter the expression and co-expression of certain genes and/or produce perturbations in signal transduction pathways that influence cellular events related to proliferation, differentiation and apoptosis [1].

Mutagenic carcinogens are classified into groups by International Agency for Research on Cancer (IARC) and Environmental Protection Agency (EPA), this classification is shown in Table 5-1.

Table 5-1 IARC and EPA classification of carcinogens

IARC	EPA	
1	Group A	Human carcinogens Sufficient evidence from epidemiological studies to support a causal association between exposure to the agents and cancer
2A	Group B	Probable human carcinogens
	Group B1	Limited epidemiological evidence that the agent causes cancer regardless of animal data
	Group B2	Inadequate epidemiological evidence or no human data on the carcinogenicity of the agent and sufficient evidence in animal studies that the agent is carcinogenic
2B	Group C	Possible human carcinogens Absence of human data with limited evidence of carcinogenicity in animals
3	Group D	Not classifiable as to human carcinogenicity Agents with inadequate human and animal evidence of carcinogenicity or for which no data are available
4	Group E	Evidence of noncarcinogenicity for humans Agents that show no evidence for carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies

Adapted from [1]

Carcinogens can be also classified, from another point of view, as chemical, biological and physical factors.

Biological agents that are causing cancer are viral, bacterial and also helminth infections. Together these agents cause estimatedly 15% of all cancers [3]. The major causative agents in world are hepatitis B virus, human papillomaviruses (HPV), *Helicobacter pylori* and human immunodeficiency virus (HIV). It is estimated that control of these diseases could prevent one of ten cancers developed [3].

Physical agents are different types of radiation. In general radiation can be divided in ionizing (with energy more than 10-15 eV; e.g. X-rays, γ-rays, neutrons and α-particles) and non-ionizing (energy less than 10 eV). The energy of radiation is imparted into biological material and can cause chemical changes having possibly biological effects. The most dangerous types of radiation are ionizing radiation and UV radiation.

Chemical agents are divided in several basic groups: inert chemicals, inorganic chemicals (e.g. heavy metals), hormone chemicals and organic chemicals. In the group of inert chemicals are sometimes included some physical agents, such as asbestos powder.

Another type of classification is into direct acting and non-direct acting agents. Non-direct acting carcinogens need the host enzymatic system and conditions to be activated to ultimate carcinogens. In metabolism of these compounds there are many steps and pathways and some of them result in detoxifying of the carcinogen while others result in activation. The process of chemically induced carcinogenesis is described in Figure 5-1.

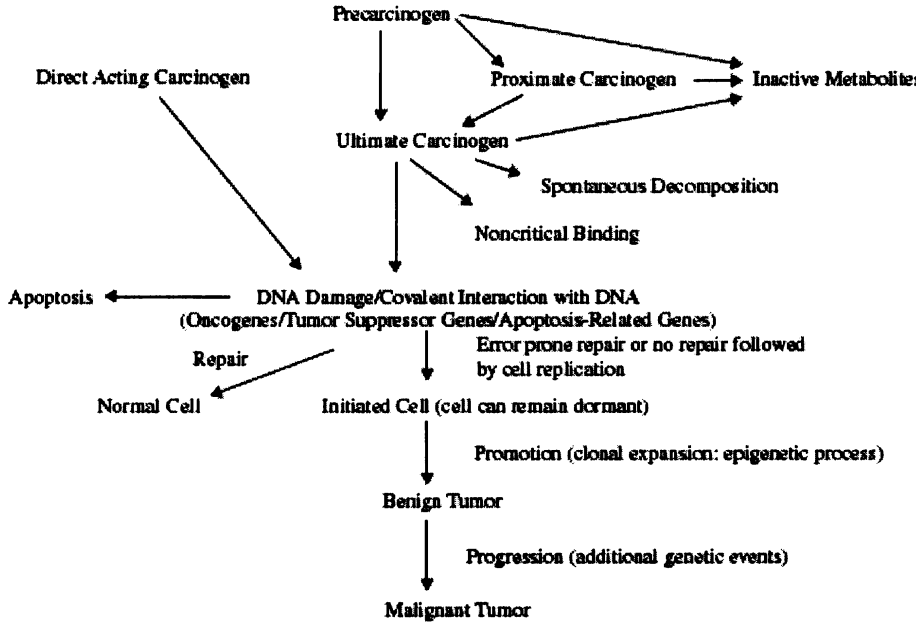


Figure 5-1 General aspects of chemically induced carcinogenesis (adapted from [1])

Organisms are continuously exposed to many of these agents, including all groups, biological, physical and chemical. The only way how to decrease the incidence of cancer caused by biological, physical and chemical direct-acting agents is in decreasing exposure to them. In the case of chemical non-direct acting carcinogens there are more possibilities, two major arise from inhibition of activation enzymes (usually phase I) and in induction of enzymes involved in detoxification of carcinogens (usually phase II).

The majority of carcinogens are non-direct acting. Hence the research of activation enzymes is one of the most important way, how to influence metabolism of procarcinogens.

People are daily exposed to many different environmental, occupational and dietary carcinogens. Dietary carcinogens are in almost all cases unconsciously taken every day in substantial amounts and because of this belong to most dangerous ones. Diet is one of three major cancer causative factors [3].

5.1 Dietary carcinogens

Almost all diet components affect the process of carcinogenesis. In general, they can be divided into macrocomponents, such as fat and sodium chloride, and microcomponents, such as mycotoxins, alkaloids, alkenylbenzenes, mushroom hydrazines, nitrosamines and nitrosatable mutagens, polycyclic aromatic hydrocarbons, heterocyclic amines and dioxins. Both of these groups have an impact on the process of carcinogenesis. Dietary genotoxins that occur in food are microbial contaminants, edible plant components, substances formed during storage and fermentation of food, products of cooking, and food additives and preservatives [3].

5.1.1 Mycotoxins

Mycotoxins are toxic compounds produced by fungi. One of the most studied mycotoxins is **aflatoxin**, produced by *Aspergillus flavus* [1, 3] and *Aspergillus parasiticus* [1] especially in humid and hot conditions. Two most abundant aflatoxins in food are aflatoxin B_1 (AFB_1) and M_1 (AFM_1). AFM_1 is produced by cow fed with AFB_1 contaminated grains and seeds, and is present in cow milk. AFB_1 is one of the most potent carcinogens, causes hepatocellular carcinomas in animals, and is classified in class 1 of carcinogens (IARC, for more see Table 5-1).

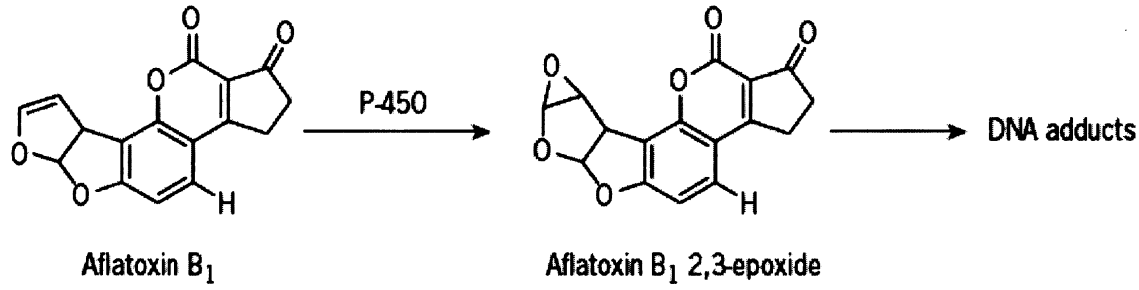


Figure 5-2 Metabolic activation of aflatoxin B_1 (adapted from [3])

Aflatoxin B_1 is metabolically activated by CYP3A4 and 1A2 forming epoxide (Figure 5-2). Following reaction is detoxifying, forming dihydrodiols, or mutagenic, forming DNA adducts. Predicted target in humans is *p53* gene [3].

Other mycotoxins occurring in food are sterigmatocystin, product of moulds in genera *Aspergillus* and *Penicillium*, which is mutagenic on *Salmonella* test strains and induces hepatomas in rats after oral administration, ochratoxin A, zearalenone, fusariums, T-2 toxin, and luteoskyrin [3, 80].

5.1.2 Heterocyclic Amines

Heterocyclic amines (HCAs) are formed in food during meat cooking from creatinine or creatine, amino acids and sugars, and are also present in cigarette smoke. Maillard reaction plays an important role in HCA formation in food, producing intermediates (e.g. pyrazines, pyridines) that are subsequently converted to HCAs through the reaction with other precursors. Amount of HCAs in cooked meat depends markedly on the composition of meat (level of creatinine, amino acids and sugars), time and method of cooking (temperature of cooking, presence of fat). The quantities of HCAs produced increase in presence of fat and with increasing temperature and time of cooking. At high temperatures, free radicals are generated through lipid peroxidation and enhance the amount of particular Maillard reaction products. The major part of HCAs is present near the surface of cooked meat. Four most abundant HCAs in cooked food are 2-amino-9H-pyrido[2,3-*b*]indole (AαC), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1,6-dimethylfuro[3,2-*b*]imidazo[4,5-*b*]pyridine (IFP) [83, 84].

All mutagenic HCAs have nitrogen within aromatic rings and exocyclic amino group and are regarded by IARC as possible (class 2B), probable (class 2A), and human carcinogens (class 1). They are classified in four major groups: imidazoazarenes, amino-α-carbolines, amino-γ-carbolines and β-carbolines. Examples of mutagenic HCAs present in food are in Figure 5-3. Target organs for HCAs are mainly liver, but also small and large intestine, oral cavity, lung, blood vessels, Zymbal gland, skin, clitoral and mammary glands [3, 87].

HCAs are activated via *N*-hydroxylation, which is predominantly catalyzed by CYP1A2, and followed by phase II enzyme reactions (conjugations), e.g. acetylation by acetyltransferase (esterification) or sulfonation by sulfotransferase, and form unstable

intermediates. They decompose and may form covalent DNA adducts [3]. HCAs are known to be competitive and non-competitive inhibitors, and inducers of CYP1A family members [82].

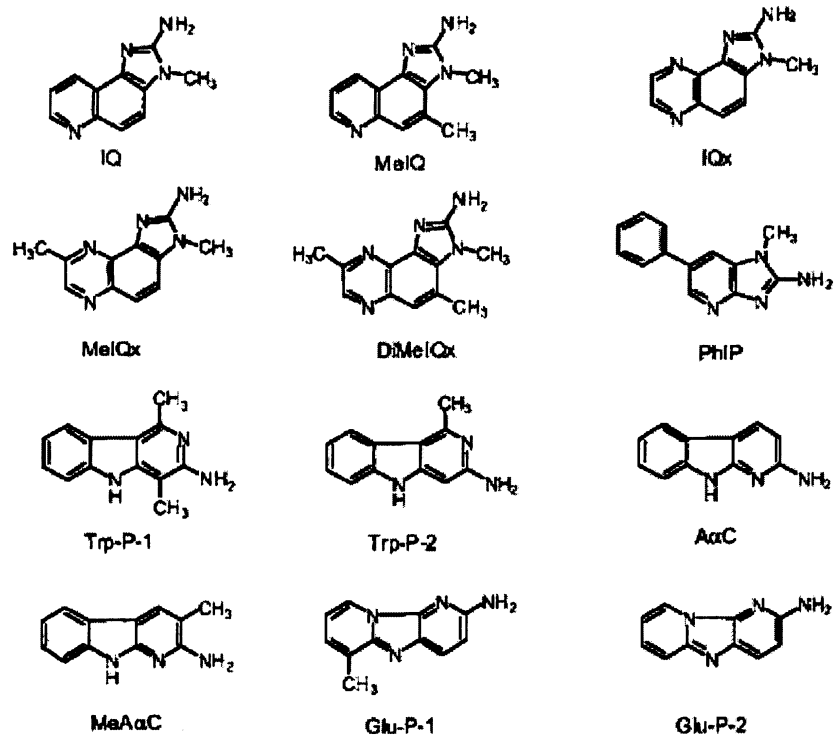


Figure 5-3 Structures of mutagenic HCAs (adapted and modified from [93])

For example, PhIP is activated by CYP1A subfamily to *N*-hydroxy derivate followed by esterification forming acetoxy or sulfoxy derivates which can spontaneously decompose forming highly reactive nitrenium ion (Figure 5-4).

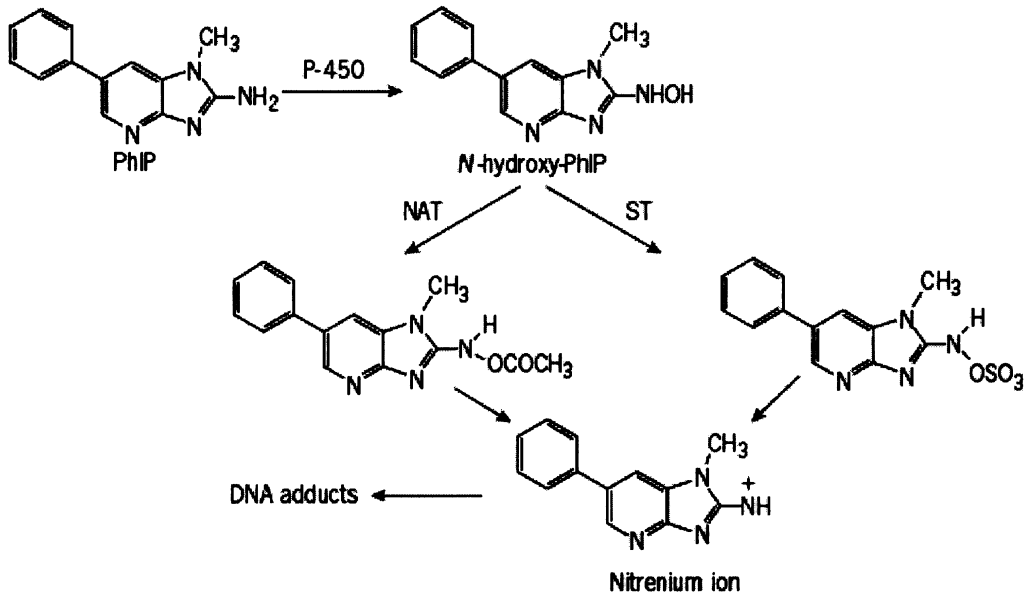


Figure 5-4 Metabolic activation of PhIP (P-450 = cytochrome P450, ST = sulphur transferase, NAT = *N*-acetyltransferase) (adapted and modified from [3])

Metabolic pathway of another HCA, MeAαC is shown in Figure 5-5.

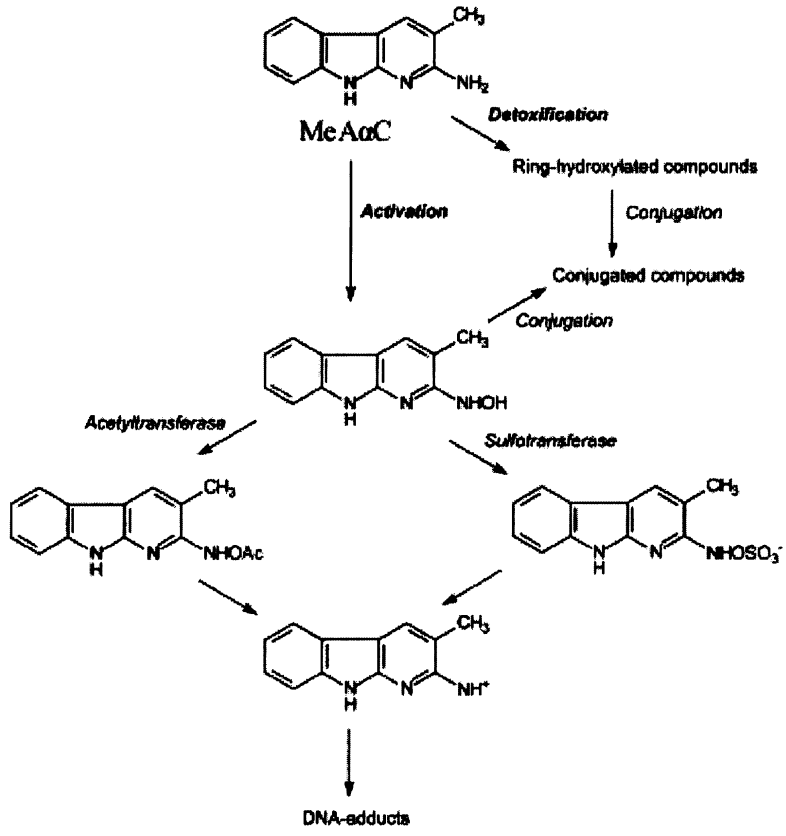


Figure 5-5 Metabolic pathway for amino-α-carbolines (MeAαC) (adapted and modified from [86])

5.1.3 *N*-Nitroso compounds

N-nitroso compounds can be divided in two groups, *N*-nitrosamines and *N*-nitrosamides. The first need metabolic activation, while the latter are direct-acting (are activated by spontaneous hydrolysis) [3].

Exposure to *N*-nitroso compounds might occur in three major ways: exogenous levels in foods (usually nitrosamines because nitrosamides are unstable), tobacco smoke, and endogenous formation (from dietary precursors). Exposure to exogenous nitroso compounds is usually from heat processed food (e.g. fried bacon, smoked fish), where nitrosamines are formed through reaction of secondary and tertiary amines with nitrosating agent (usually nitrous anhydride formed from sodium nitrite – used food preservative and colouring substance in food). Level of nitrosamines is increased by intake of nitrates, which are transformed to nitrite by gastrointestinal microflora. Intake of nitrates is usually from vegetable while intake of nitrites is usually from cured meat. The formation of nitrosamines during cooking is temperature-dependent, and the highest amounts of NPYR, one of the most potent nitrosamine carcinogens, are formed at high temperature [90].

Precursors of endogenously formed nitrosamines in food are primary amines (e.g. methylamine from cheese, pickles, vegetable, and tyramine or tryptamine from meat and cheese), secondary amines (e.g. dimethylamine from fish, and pyrrolidine), amino acids, aromatic amines (e.g. *N*-methylaniline from cheese), guanidines (e.g. creatine from meat), and ureas (e.g. citrulline from fruit and vegetable). *N*-nitrosamines are formed endogenously by nitrosation of substituted amides, ureas, carbamates and guanidines [11]. Moreover reactive nitric oxide, produced from L-arginine by nitric oxide synthetase in inflammatory processes, is involved in the generation of *N*-nitroso compounds. For example *N*-nitrosodialkylamines are formed by reaction of nitrite with secondary amines in acidic conditions in stomach. Nitrite can also react with indole and phenol derivatives (nitrosatable) in acidic conditions forming mutagenic compounds and can react forming diazo compounds.

Most abundant HCAs in food are *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosopiperidine [90].

N-nitrosamines are activated via 2-hydroxylation catalyzed by cytochrome P450s (mainly CYP2E1 and CYP2B1). Shu and Hollenberg [89] investigated the substrate specifity of these enzymes. CYP2B1 is predominantly involved in activation (dealkylation) of *N*-nitrosodipropylamine (NDPA), *N*-nitrosobutylpropylamine (NBPA) and *N*-nitrosodibutylamine (NDBA), while CYP2E1 is involved in dealkylation of NDPA and NDBA.

N-Nitrosodimethylamine (DMNA) is usually used as a representative member of nitrosamines. This compound is hepatotoxic, mutagenic and carcinogenic. In DMNA activation CYPs are involved causing *N*-demethylation to form *N*-methylnitrosamine. Then spontaneously methyldiazonium ion and ultimately methyl carbonium, ion which is highly reactive alkylating agent, is formed (Figure 5-6).

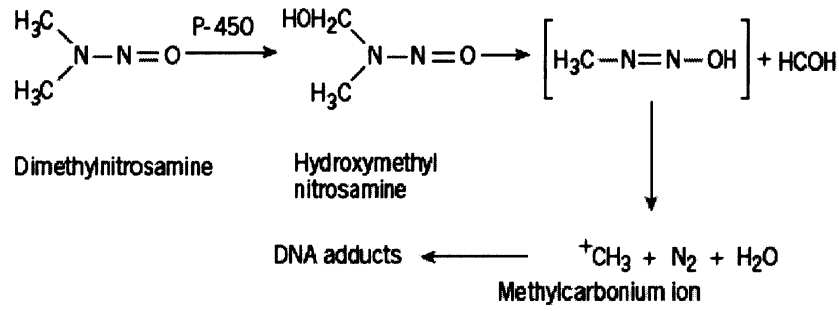


Figure 5-6 Metabolic activation of *N*-nitrosodimethylamine (P-450 = cytochrome P450) (adapted and modified from [3])

5.1.4 Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in environment (e.g. cigarette smoke, exhaust gas) and in cooked food (e.g. charcoal-broiled meat). These compounds consist of at least two condensed aromatic rings. In general, activated PAHs that are epoxidized in the "bay region" possess carcinogenic properties. PAHs are also responsible for induction of activation enzymes (mainly CYP1A) and because of this, dietary PAH exposure can enhance the susceptibility to subsequent PAH (and other carcinogen) metabolic activation.

PAHs are metabolically activated mainly by CYP1A1 and CYP1B1 to epoxide intermediates that are further converted to more reactive diol-epoxides by epoxide hydrolase [80]. In the presence of nitrite in acidic conditions, PAHs are converted to direct-acting, highly mutagenic nitro-derivatives [81].

Benzo(a)pyren (BaP) is usually used as an example of PAHs. In studies at least 15 metabolites have been identified as products of CYP1A1 and epoxide hydrolase catalyzed reactions [1]. Many of these metabolites are substrates for phase II enzymes (detoxification pathway). On other hand BaP can be activated into the ultimate diol epoxide derivate (ultimate carcinogen) [1, 3], oxide or dihydrodiol (proximate carcinogens) [1]. Metabolic activation of BaP is shown in Figure 5-7.

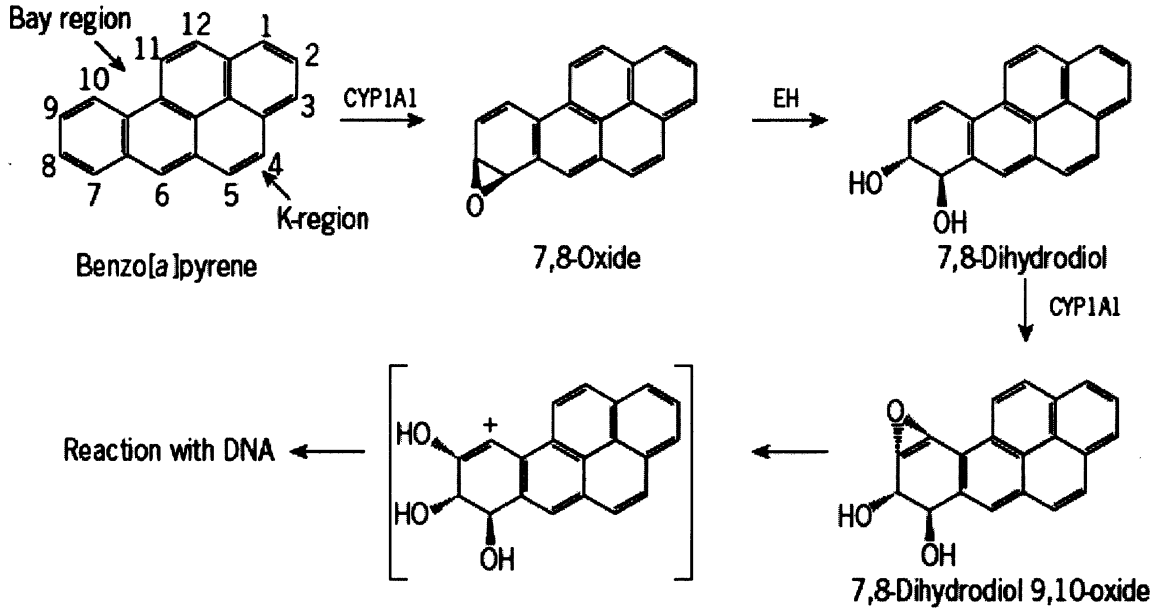


Figure 5-7 Metabolic activation of benzo(a)pyren (P-450 = cytochrome P450, EH = epoxide hydrolase) (adapted from [3])

6 Chemoprevention

Many chemical compounds naturally present in plants where found to have beneficial effects on human and animals. Natural chemopreventive or synthetic non-essential dietary agents have potential to interfere with the process of carcinogenesis and prevent or delay tumour growth. Chemoprevention is defined as a daily use of dietary constituents to prevent mutagenesis and carcinogenesis.

In chemoprevention there are three main strategies, first preventing cancer in healthy high risk individuals, second preventing development of cancer in individuals with precancerous lesions, and third preventing development of secondary primary tumour or recurrence in patients who have had cancer previously [10].

Carcinogenesis is influenced also by many behaviour factors, e.g. diet and physical activity. Composition of diet is responsible for about 30 percent of cancer risk, in major the risk of gastrointestinal tract cancer. Dietary factors can be beneficial (positively modulate molecular targets of carcinogenesis) or negative, e.g. carcinogenic. Modifying diet is an important way how to reduce incidence of cancer.

Recent research in this area is focused on developing new chemopreventive compounds based on known molecular mechanism of carcinogenesis. Intake of these agents would be beneficial and could decrease the incidence of cancer.

Preventive dietary factors can be classified into three major groups [3]:

- agents that prevent formation of carcinogens,
- agents that inhibit carcinogenesis, prevent carcinogen reaching critical targets (also called blocking agents), and
- agents acting subsequently to exposure to carcinogen (act after initiation, also called suppressing agents).

There is another, fourth group of dietary chemopreventive compounds which cannot be included in first three mentioned. These involve non-steroidal anti-inflammatory compounds (NSAIDs) because of connection of cancer with inflammation [10].

Antigenotoxins, compounds that have potential to interrupt the process of mutagenesis, can be endogenous (direct acting simple compounds or complex molecules, e.g. enzymes involved in detoxification of reactive metabolites) and exogenous (synthetic or naturally occurring, used as food additives) and can act for example as radical scavengers or antioxidants.

Naturally occurring chemopreventive compounds might be also classified from another point of view, their chemical structures. These groups are:

- vitamins (vit. A, C, E, B_2, B_6, B_{11}, B_{12}),
- minerals and trace elements (Ca, P, Zn, Se, Mb), and
- other compounds of plant origin:
  - sulphides from allium vegetables (onion, garlic, chives),
  - dithiolthiones and glucosinolates (converted into isothiocyanates and indoles) from cruciferous vegetable (cauliflower, cabbage, Brussel sprouts),
  - terpenoids (plus limonene) from citrus fruit,
  - phyto-oestrogens (isoflavones and lignans) from cereals and pulses, wholegrain products, seed, fruit and berries,
  - flavonoids (e.g. quercetin, kaempherol, rutin, tangeritin, myricetin) from fruit and vegetable (e.g. berries, tomatoes, onion, broccoli, beans, citrus fruit), and
  - phenolic compounds (ellagic acid, caffeic acid, ferulic acid, resveratrol) from nuts, fruit, wine and tea.

6.1 Mechanisms of chemoprevention

Chemopreventive compounds influence the whole process of carcinogenesis at various stages:

- initiation by inhibition of activation enzymes or enhancement of detoxifying enzymes and/or radical scavengers,
- promotion by inhibition of DNA synthesis, cell proliferation and alteration of cell differentiation and communication, and inhibition of signal transduction pathways,
- and progression by inhibition of cell proliferation.

As mentioned above, chemopreventive compounds can be classified into three groups: inhibitors of carcinogen formation, blocking agents (affect the initiation phase) and suppressing agents (affect the promotion and progression phases). Examples of these agents are shown in Table 6-1, and the target sites of chemopreventive agents are shown in Figure 6-1.

Table 6-1 Classification and examples of chemopreventive agents

Category of inhibitor	Chemical class	Inhibitory compound
Compounds preventing formation of carcinogen	Reductive agents	Vitamin C[a]
	Tocopherols	α-Tocopherol[a], γ-tocopherol[a]
	Phenols	Caffeic acid[a], ferulic acid[a], gallic acid[a]
Blocking agents	Phenols	t-Butylhydroxyanisole[b], butylated hydroxytoluene[b], ellagic acid[a], caffeic acid[a], ferulic acid[a]
	Indoles	Indole-3-acetonitrile[a], indole-3-carbinol[a]
	Coumarins	Coumarin[a], limettin[a]
	Flavones	Quercetin[a], rutin[a], catechin[a]
	Aromatic isothiocyanates	Benzyl isothiocyanate[a], phenyl isothiocyanate[a]
	Dithiolthiones	Oltipraz[b]
Suppressing agents	Retinoids and carotenoids	Retinyl palmitate[a], retinyl acetate[a], β-carotene[a]
	Protease inhibitors	Soybean protease inhibitors[a]
	Inhibitors of arachidonic acid metabolism	Indomethacin[b], aspirin[b]
	Phenols	t-Butylhydroxyanisole[b],
	Methylated xanthines	Caffeine[a]
	Plant sterols	β-Sitosterol[a]
	Selenium salts	Sodium selenite[a], selenium dioxide[a], selenious acid[a]

[a]Naturally occurring compound present in food or formed during digestion.
[b]Synthetic compound.

Adapted from [3]

Inhibitors of carcinogen formation are trapping (scavenging) precursors of endogenously formed carcinogens so that they cannot be created. These agents are for example ascorbic acid (prevents endogenous formation of *N*-nitroso compounds), vitamin E (prevents the formation of nitrosamines by scavenging nitrates), ferulic acid, gallic acid, caffeic acid, *N*-acetylcysteine, proline and thioproline.

Blocking agents prevent the carcinogens from reaching and reacting with critical targets, such as DNA. In the case of non-direct acting carcinogens, they are interfering with their metabolism, involving modulation of phase I and phase II xenobiotic metabolizing enzymes.

These agents can be further divided into few groups [3]:

- inhibitors of CYPs – they reduce the CYP activation of procarcinogens (e.g. ellaic acid, diallyl sulphide, isothiocyanates),
- inductors of CYPs (e.g. indole-3-carbinol) – these inductors enhance the formation of such compounds that are subsequently detoxified by phase II enzymes,
- inductors of phase II enzymes, such as glutathione *S*-transferases (inducible by e.g. vitamins, indole-3-carbinol, limonene, phenyl isothiocyanate) and

glutathione peroxidases (selenium dependent or independent), which can enhance the detoxification of carcinogens,

- scavengers of electrophiles and free radicals (e.g. natural antioxidants such as vitamins C and E, carotenoids and flavonoids) – prevent damaging DNA and other molecules, and
- inducers of DNA repair system – result in increase of overall DNA repair enzymes level (e.g. vanillin), inhibition of proteases that activate error-prone repair system (e.g. soy bean trypsin inhibitors, found in bacteria), stabilization of poly(ADP-ribosyl) transferase level (carcinogen usually decrease the level of this enzyme, e.g. *N*-acetylcysteine).

Suppressing agents act after initiation cell is formed. These agents were classified by Keloff [3] into several groups:

- inhibitors of polyamine metabolism (e.g. polyphenols, ellagic acid, curcumin) – levels of polyamine and ornithin decarboxylase are elevated in tumour cells stimulating or maintaining high proliferation rates,
- inducers of terminal cell differentiation (e.g. calcium, vitamin D_3, retinoids) – tumour cells have usually lost their ability to differentiate and these compounds restore this ability,
- modulators of signal transduction (e.g. flavonoids, glycyrrhentinic acid, retinoids),
- modulators of hormonal and growth factor activity (e.g. retinoids) – they directly regulate the levels and biological activities of specific hormones and growth factors (e.g. competing for binding sites to their receptors),
- inhibitors of oncogene activity/expression (e.g. genistein, retinoic acid, monoterpens),
- promoters of intercellular communication (e.g. carotenoids, polyphenols, retinoids) – they enhance the gap junctional communication (usually inhibited in tumour cells) and inhibit cell transformation,
- restorers of immune response (e.g. selenium, vitamin E, retinoids) – they stimulate the elimination of transformed and otherwise abnormal cells,
- inducers of apoptosis (e.g. genistein, selenium, retinoids),
- correctors of DNA methylation imbalances (e.g. folic acid, choline, methionine, vitamin B_{12}),

- inhibitors of basement membrane degradation (e.g. protease inhibitors) – cancer cells contain several enzymes that digest basement membrane allowing invasion through normal tissue, these agents act as inhibitors of this process, and
- inhibitors of arachidonic acid metabolism (e.g. glycyrrhetic acid, flavonoids, *N*-acetylcysteine, vitamin E) – from arachidonic acid are formed many reactive compounds, such as reactive oxygen species, these agents eliminate their formation.

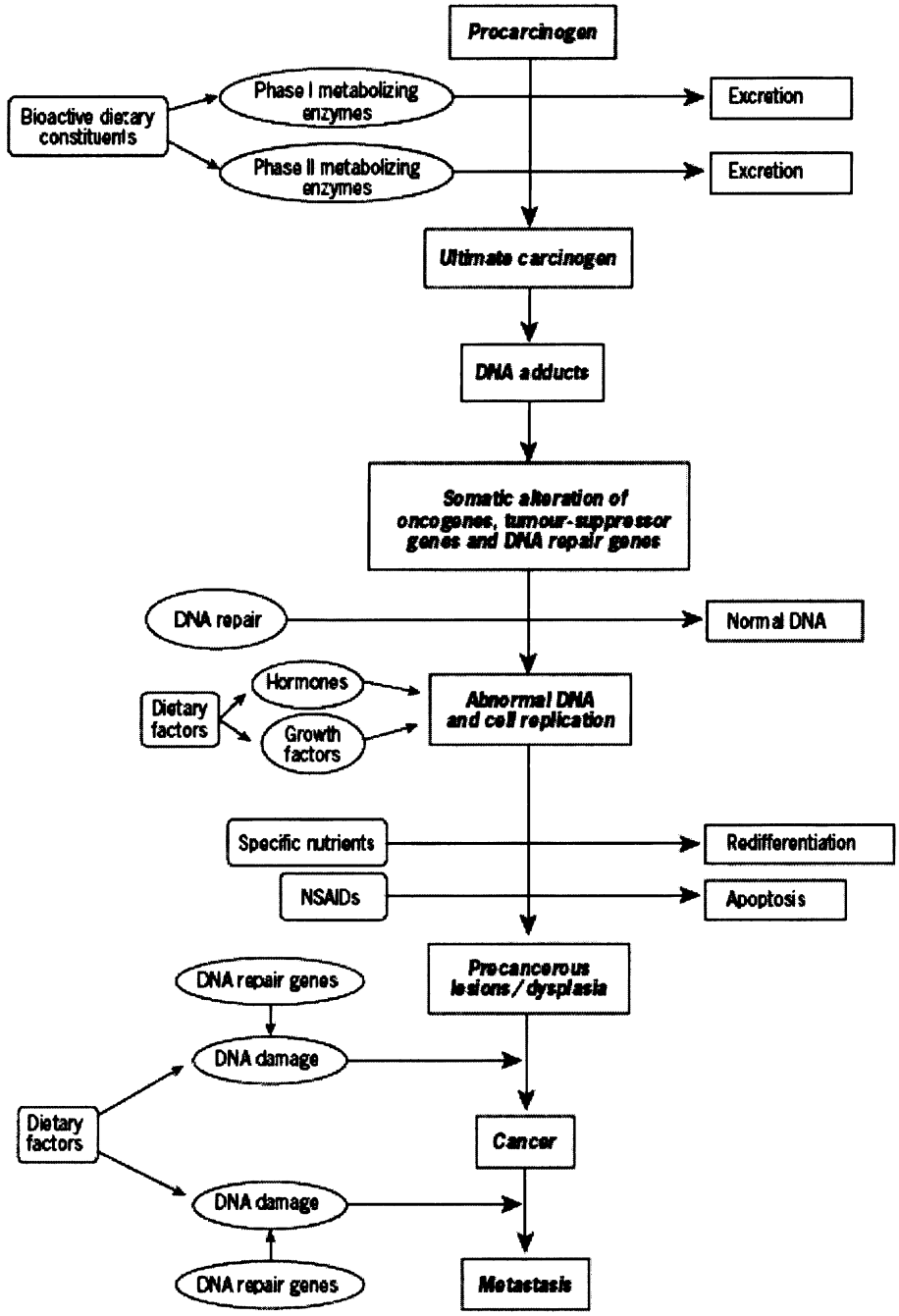


Figure 6-1 Target sites of antimutagens and anticarcinogens in carcinogenesis (adapted from [3])

6.2 Plant chemopreventive compounds

Higher plants contain variety compounds which are strong modifiers of carcinogen metabolism and chemical carcinogenesis. More than 2000 natural and derived synthetic compounds (analogues of natural molecules) are known to have chemopreventive effects and are under intensive research.

6.2.1 Phenolic compounds

These compounds are present in various plants, and have antimutagenic and anticarcinogenic potential, modulate various enzymes and cell receptors, and are metabolized mainly by intestinal and hepatic enzymes and intestinal microflora. Phenolic compounds were found to have antioxidant properties, and have potential to prevent various diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases.

These compounds can be classified into four groups by number of aromatic rings and structural elements that bind these rings to another one(s): phenolic acids, flavonoids, stilbenes and lignans. Structures of these compounds are shown in Figure 6-2. These compounds can be conjugated with various carbohydrates, organic acids, etc.

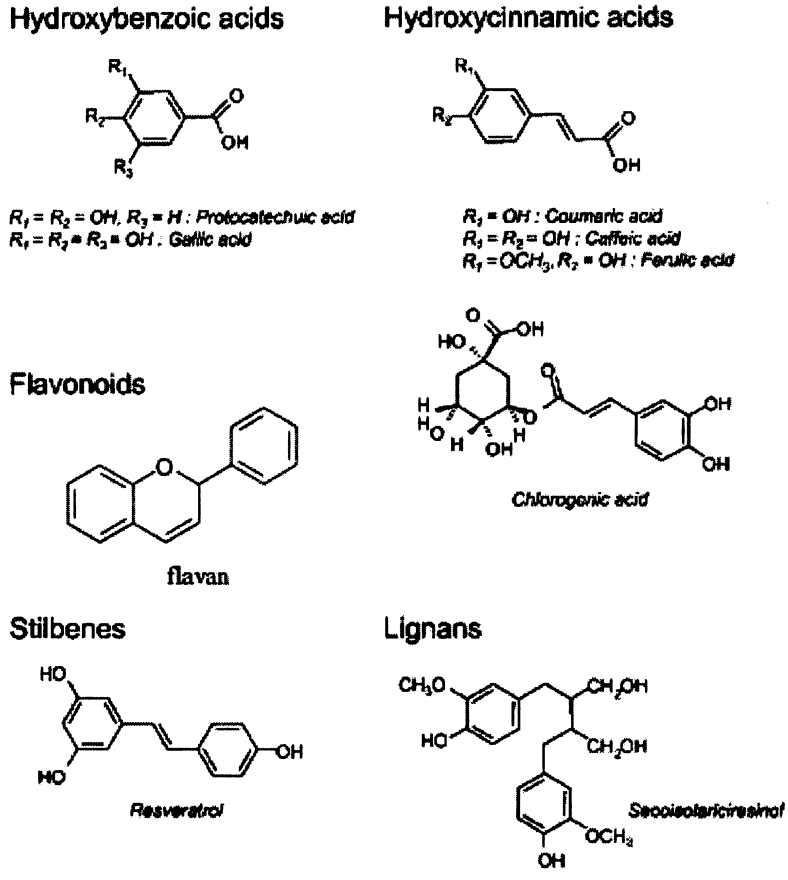


Figure 6-2 **Structures and examples of phenolic compounds (adapted and modified from [12])**

Phenolic acids

Phenolic acids are derivatives of benzoic and cinnamic acid. Hydroxybenzoic acids are present in low content in red fruits, black radish, onion, and tea (gallic acid), and are components of hydrolysable tannins. These acids are present free or esterified. Most common group of phenolic acids is hydroxycinnamic acids, for example *p*-coumaric, caffeic, ferulic and sinapic acid. These are rarely found free (only in processed food) and are usually found as glycosides and esters of quinic acid, shikimic acid and tartaric acid, and are present in blueberries, kiwi, plums, cherries and apples.

Flavonoids

Flavonoids are based on the structure of flavane (Figure 6-2), and can be divided into few groups according to their further structure (Figure 6-3): flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols.

In general, flavonoids have anti-bacterial, anti-viral, anti-inflammatory, antioxidant, anti-angionic, analgesic, anti-allergic, hepatoprotective, cytostatic, apoptotic, estrogenic and anti-estrogenic properties. All these activities are beneficial, but flavonoids exert also some negative properties, such as mutagenic (e.g. quercetin [29,57]), and pro-oxidative [27]. Flavonoid compounds are involved in interaction with CYPs in at least three ways: induce biosynthesis of several CYPs, modulate CYP activities, and are metabolized by CYP [27]. They also modulate activity of phase II enzymes, usually enhance activity of these enzymes.

As flavonoids are present in food mainly as glycosides, they cannot be absorbed directly after ingestion but are hydrolyzed in the lower part of intestine to their aglycones by bacterial enzymes. With exception of anthocyanins (mainly present in food as glucosides) which appear to be absorbed in a limited extent after oral ingestion [reviewed in 26].

- **Flavonols** are present in food in higher amounts than other flavonoids. The most abundant are quercetin and kaempferol. Flavonols are present in onion, curly kale, leeks, broccoli, blueberries, red wine and tea (mainly in outer parts, which are exposed to sunlight), and are found mainly as glycosides with sugar moiety.
- **Flavones** mainly occur as glycosides of luteolin and apigenin, are present in parsley and celery, in cereals (wheat and millet) and in citrus fruit skin (polymethoxylated flavones: tangeretin, nobiletin, sinesetin).

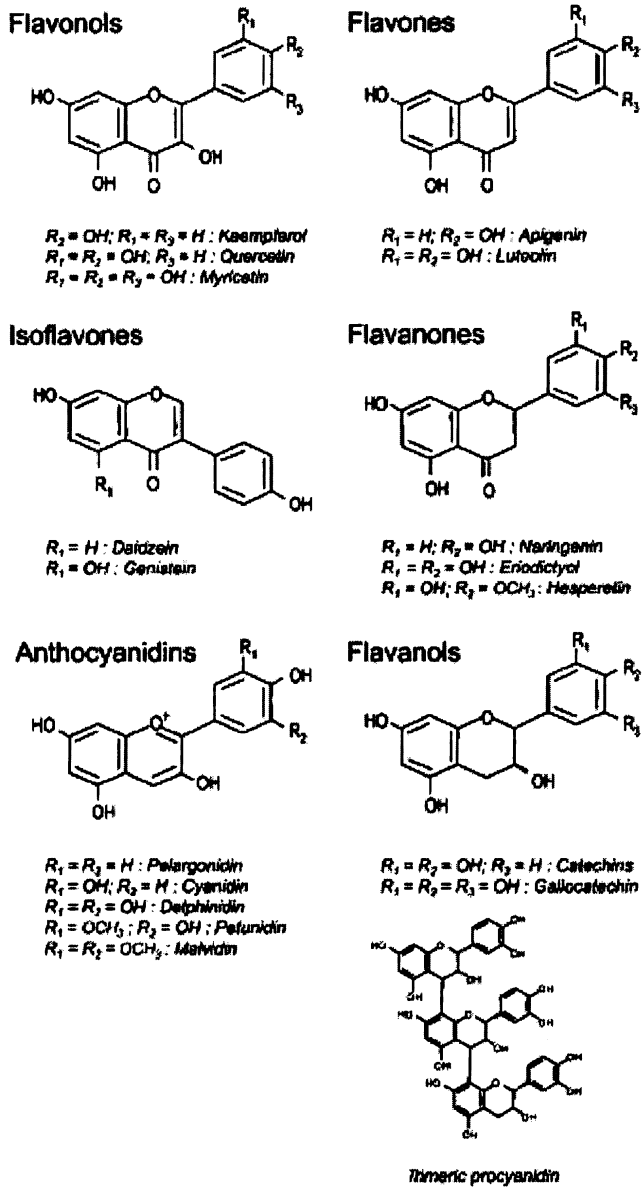


Figure 6-3 Chemical structures and examples of flavonoids (adapted from [12])

- **Flavonones** are present in tomatoes and aromatic plants (e.g. mint), and in highest concentration in citrus fruit (as aglycones: e.g. naringenin, hesperetin, eriodictyol). Flavonones are usually glycosylated by disaccharids.

- **Isoflavones** are also called phytoestrogens because of their structural similarities to estrogens and their pseudohormonal properties. The most frequent isoflavones are genistein, daidzein and glycitein, and are present in leguminous plants.

- **Flavanols** naturally occur as monomers (catechins) and polymers (proanthocyanidins). Catechins are usually not glycosylated and are present as aglycones in apricots, red wine, chocolate, and green tea. The most abundant in fruit are catechin and epicatechin, while in seed of leguminous plants, grapes and tea are gallocatechin,

epigallocatechin and epigallocatechin gallate. Proanthocyanidins, which are also known as condensed tannins, are dimers, oligomers and polymers of catechins that are bound together by links between C4 and C8 (or C6). An example of proanthocyanidins is shown in Figure 6-3. In black tea are present dimers (theaflavins) and polymers (thearubigins) of catechin.

- **Anthocyanins** are water soluble vacuolar flavonoid pigments that reflect the red to blue range of the visible spectrum, depending on the pH of the surrounding solution. They can be subdivided into the sugar-free anthocyanidine aglycones (highly unstable in light and oxidation conditions) and the anthocyanin glycosides. In plants they are usually protected from degradation by glycosylation, mainly with glucose, and esterification with various organic acids (citric and malic acids) and phenolic acids. In addition, anthocyanins are stabilized by the formation of complexes with other flavonoids (copigmentation). In the human diet, anthocyanins are found in red wine, certain varieties of cereals, and certain leafy and root vegetables (aubergines, cabbage, beans, onions, radishes), but they are most abundant in fruit. Cyanidin is the most common anthocyanidin in foods.

Chemopreventive properties of flavonoids

The study of Catterall et al. [60] investigated the influence of flavonoids isolated from grape seeds, almond fruits, apple skin and green tea on genotoxicity of food carcinogens by Ames test of mutagenicity. Neither the monomeric nor dimeric polyphenolic compounds and their galloyated derivatives influenced the activity of BaP, IQ and *N'*-methyl-*N'*-nitronitrosoguanidine (MNNG). In contrast, all used flavonoids enhanced the activity of *N*-nitrosopyrrolidine (NPYR).

Hirose et al. [68] investigated effect of some natural and synthetic phenolic antioxidants on MeIQx associated hepatocarcinogenesis. Synthetic phenolics, such as 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (*t*-BHQ), propyl gallate and troglitazone, were much more effective than natural, such as ferulic acid, chlorogenic acid, curcumin, green tea catechins, hesperidin, quercetin, rutin, daidzin and genistein, in the quantitative analysis of the placental form of glutathione-S transferase (GST-P) foci formation and growth, the most effective was HTHQ. From all natural phenolics, only green tea catechins inhibited MeIQx carcinogenity, others even enhanced GST-P positive foci development. All these antioxidants interfered with CYP1A2 activity and slightly decreased CYP1A1

activity. Treatment with HTHQ for 2 weeks increased CYP1A2, CYP2B1/2 levels in rat liver.

In the study of Sun and Fukuhara [72] butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) were co-administrated with flavone or flavanone for 2 weeks to male mice and the ability of liver microsomes to activate AFB_1, BaP and *N*-nitrosodimethylamine (NDMA) was measured. When these mixtures were given to mice, activation of BaP and AFB_1 was enhanced while NDMA activation did not increase significantly. Co-administration of BHT and flavone markedly increased the protein levels of CYP2A and CYP2B, and co-administration of BHA and flavanone increased the protein level of CYP1A. Mixture of BHA and BHT was reported to modulate phase II enzyme activity, mainly glutathione *S*-transferase (GST).

Modulation of cytochromes P450 by flavonoids

At low concentration, flavonoids act as inhibitors of CYP activity, but at higher concentrations, some of them act also as inducers of CYP, usually elevating expression of CYP genes, mainly *CYP1A1/2* and *CYP2B1/2*. Influence of particular flavonoids on CYP activities are shown in Table 6-1.

Study of Ueng et al. [34] observed tissue-specific effect of wogonin and baicalein on CYP. In C57BL/6J mice both baicalein and wogonin modulated CYP activities and CYP levels. In liver baicalein and wogonin decreased levels of CYP2E1 and CYP3A4, but effect on CYP1A levels was different. Baicalein increased level of CYP1A2 and wogonin decreased this hepatic CYP level, while in lung both of them increased levels of CYP1A. Another study of Ueng et al. [35] is focused on the influence of these flavonoids on AFB_1 and BaP induced genotoxicities. AFB_1 is metabolically activated by CYP1A2 and CYP3A4, and there could be a possible negative interference of baicalein and AFB_1 metabolism because of CYP1A2 modulation.

Lignans

Lignans contain two phenylpropane units, example is shown in Figure 6-2. The richest dietary source of lignans is linseed, which contain for example secoisolariciresinol and matairesinol. Other sources in human diet are also cereals, grains, fruit and certain vegetables, but these plants contain much lower concentrations of lignans.

Table 6-2 Modulation of cytochrome P450 by phenolic compounds

CYP enzyme	Inducers	Inhibitors	no effect
1A1	Quercetin [27]	Quercetin [27,28]	Genistein [27]
	Galangin [27]	Galangin [27]	Equol [27]
	Diosmin [27]	Kaempferol [27]	Prenylchalcones [27]
	Diosmetin [27]	α-Naphtoflavone [29]	Prenylflavanones [27]
	Tangeretin [27,28]	Ellagic acid [29]	Flavanone [28]
	Flavone [27,28]	Capsaicin [29]	
	β-Naphtoflavone [27]	Resveratrol [24,25,29,48]	
	Apigenin [29]	Propyl gallate [68]	
	Propyl gallate [68]	Diosmin [48	
1A2	Flavone [27]	α-Naphtoflavone [29]	Genistein [27]
	Tangeretin [27,28]	Resveratrol [24,25,48]	Equol [27]
	β-Naphtoflavone [27]	Wogonin [34]	Flavanone [28]
	Baicalein [34]	Diosmin [48]	
	HTHQ [68]		
2B1/2	Flavanone [27,28]	Capsaicin [29]	Quercetin [28]
	Flavone [27,28]	Resveratrol [24,25,48]	
	Tangeretin [27,28]		
	HTHQ [68]		
2E1		Ellagic acid [29]	Flavone [28]
		Capsaicin [29]	Flavanone [28]
		Resveratrol [25]	Tangeretin [28]
		Baicalein [34]	Quercetin [28]
		Wogonin [34]	
3A4	α-Naphtoflavone [27,29]	Naringenin [29]	Flavone [28]
		Resveratrol [25]	Flavanone [28]
		Baicalein [34]	Tangeretin [28]
		Wogonin [34]	Quercetin [28]

Stilbenes

Stilbenes are found in low concentration in human diet. Resveratrol (3,5,4'-trihydroxystilbene) is a phytoalexin consumed in human diet in high amounts. It is present in mulberries, peanuts and grapes [24,25]. Stilbenes are produced in plants in response to pathogen attack, UV irradiation and exposure to ozone [24]. For example resveratrol is synthesized by grapes in response to fungal infections.

Resveratrol (Figure 6-4) has been reported to have antioxidative, anticoagulative, anti-inflammatory, cell growth-modulatory, cardioprotective and anticarcinogenic properties [reviewed in 24]. Because of this, resveratrol was suggested as a

chemopreventive agent inhibiting diverse cellular events associated with tumour initiation, promotion and progression. *In vitro*, resveratrol affects the activity of CYP (inhibits CYP1A and CYP2B1/2 [24,25], CYP3A4 and CYP2E1 [25], and is one of the most selective inhibitors of human CYP1A1), and induces phase II enzymes (e.g. NAD(P)H quinone oxidoreductase), scavenge reactive oxygen species and block production of carbon a nitrogen-centered free radicals [reviewed in 24].

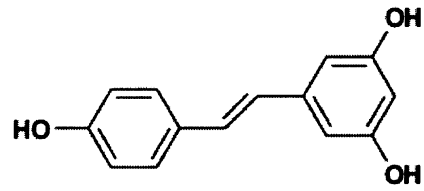


Figure 6-4 Chemical structure of resveratrol

Resveratrol is metabolized into piceatannol (3,5,3',4'-tetrahydroxystilbene) and another tetrahydroxystilbene named M1 (proposed to be 3,4,5,4'-tetrahydroxystilbene). These metabolites are shown in Figure 6-4. This reaction is catalyzed mainly by CYP1B1 and CYP1A2 (in human liver microsomes and *in vitro* by recombinant CYPs) [25].

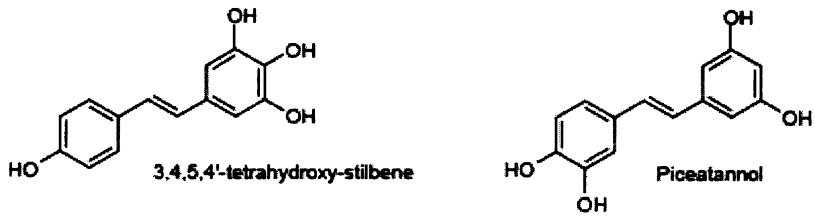


Figure 6-5 Chemical structure of metabolites of trans-resveratrol: 3,4,5,4'-tetrahydroxystilbene and piceatannol (3,5,3',4'-tetrahydroxystilbene)

6.2.2 Isothiocyanates

Isothiocyanates normally occur in cruciferous vegetable (*Brassicaceae*, e.g. broccoli, cabbage, Brussel sprouts) as thioglucoside conjugates, called glucosinolates. Isothiocyanates are produced by hydrolysis from these glucosinolates. This reaction is catalyzed by enzyme myrosinase (is present in some cell compartments of this vegetable and is released when these compartments are damaged), by intestinal microflora or cooking. Synthesis of isothiocyanates and metabolism of glucosinolates are shown in Figure 6-6.

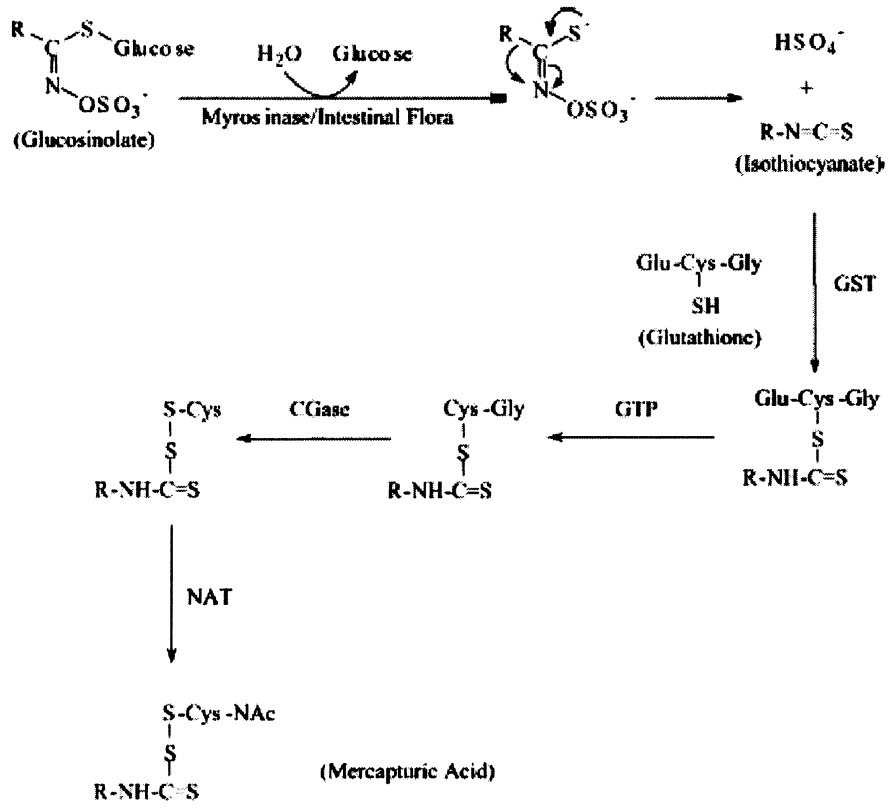


Figure 6-6 Synthesis and metabolism of isothiocyanates (GST = glutathione *S*-transferase; GTP = γ-glutamyltranspeptidase; CGase = cysteineglycinase; NAT = *N*-acetyltransferase) (adapted from [23])

Isothiocyanates modulate carcinogen metabolism, usually as blocking agents by inhibition of activation enzymes or/and enhancement of detoxifying enzymes [13]. Moreover, they can act as suppressing agents by induction of apoptosis and inhibition of cell cycle progression [22].

The inhibitory effect of these compounds on carcinogenesis was observed, e.g. when carcinogens, such as NNK, BaP, NBMA and DMBA, were administrated with some isothiocyanates. On the contrary, few other studies show the enhancement of carcinogenesis, e.g. azoxymethane (AOM) and nitrosobezylmethylamine (NBMA) co-administrated with $Ph(CH_2)_6$-N=C=S. Isothiocyanates themselves exert also some toxic properties, because they directly alkylate and deplete cellular thiols, damage mitochondria, and elevate reactive oxygen species levels (leading to oxidative stress). But there is also some evidence that isothiocyanates are more toxic to tumour cells than to normal cells [22].

Effects of isothiocyanates are widely studied in connection with lung cancer induced by tobacco smoke.

6.2.3 Indole-3-carbinol

Indole-3-carbinol (I3C) is a degradation product of glucobrassicin, compound present in cruciferous vegetable (Brassica plants), and is transformed by acidic environment in stomach into dimers, polymers and other derivatives, e.g. diindolylmethane (dimer), and indolo[3,2-*b*]carbazole.

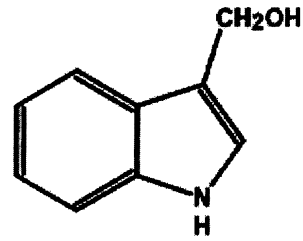


Figure 6-7 Chemical structure of indole-3-carbinol

I3C has anticarcinogenic properties, modulates metabolism of certain carcinogens (non-direct acting), such as BaP, NNK and AFB_1, but have no effect on direct-acting carcinogen-induced carcinogenesis. These effects suggest the mechanism of chemoprevention as a modulator of activation (phase I, e.g. CYP) and detoxification (phase II enzymes, e.g. glutathione *S*-transferase) enzymes activity [20]. In some animal models, I3C exert inhibitory effect at the initiation stage of carcinogenesis, e.g. prevent carcinogen-DNA binding in trout and rats, and at the promotional stage, e.g. inhibit cell growth [reviewed in 20].

Orally administrated I3C induces CYP1A1, 1A2, 2B1/2 and 3A1 activities in female rat liver. Horn et al. [21] measured cytochrome P450 content and activity (by EROD, MROD, BROD and PROD), and level of mRNA by RT-PCR methods (Figure 6-8). The increase of CYP activity seems to be dose and time dependent. While the activity increases with amount of I3C administrated, the time dependent curve is different. Activity and mRNA levels of CYPs were lower after 10 days of repeated administration than after 4 days (Figure 6-8). Enhancement of mRNA levels was only small, but the activity of cytochromes increased markedly. Most extensive increase was observed in CYP1A1 (measured by EROD) and CYP2B1 (measured by BROD) activity.

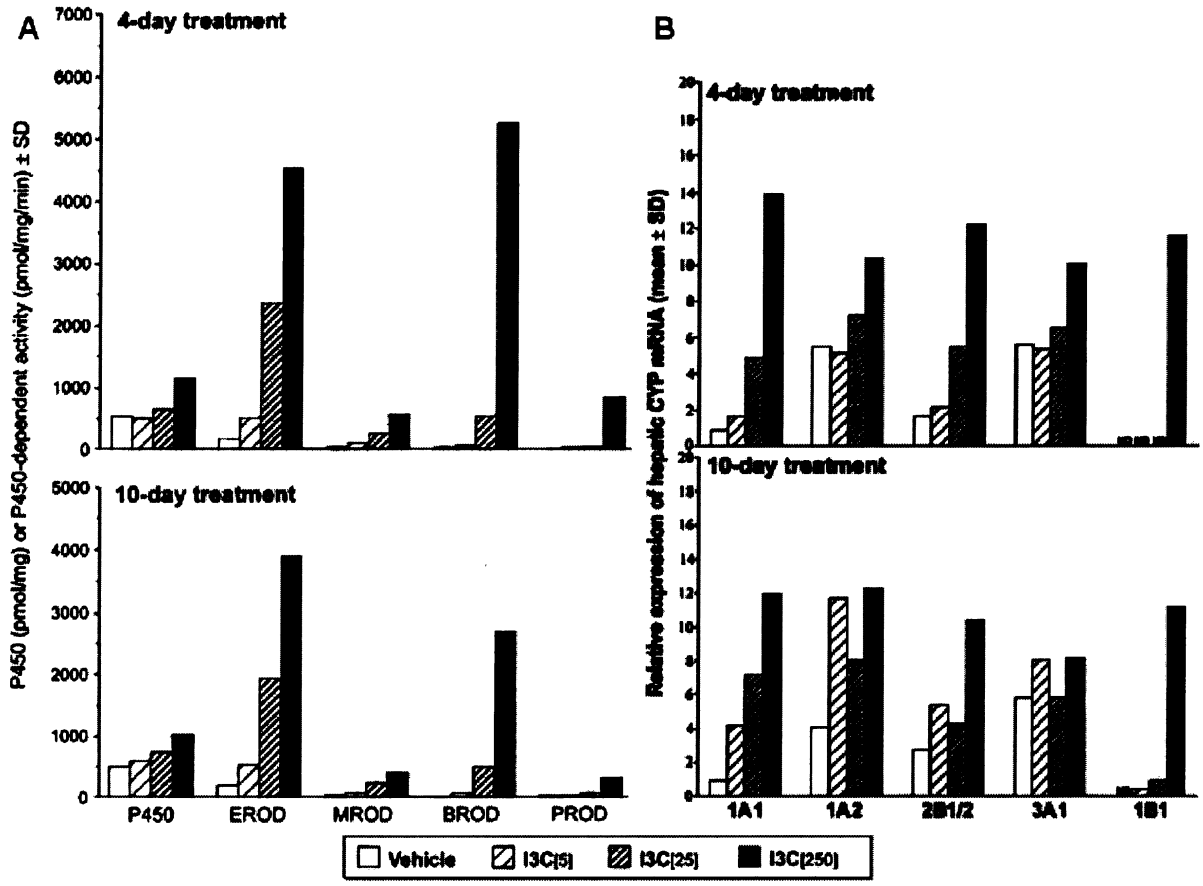


Figure 6-8 Effect of I3C treatment on A, CYP activities and CYP content, B, hepatic CYP mRNA expression; values in brackets denote the dose level (mg/kg body weight) of I3C administered by oral gavage, (adapted and modified from [21])

6.2.4 Organosulphur compounds

Organosulphur compounds (OSC) can be divided into water-soluble (e.g. *S*-methylcysteine, *S*-allylcysteine) and oil-soluble (e.g. diallyl sulphide). These compounds are present mainly in garlic and onion. Alliin (*S*-allylcysteine sulphoxide), the prime organosulphur compound present in garlic, is unstable and forms many oil-soluble organosulphur compounds through the action of enzyme allinase, cooking or by metabolism in animals [15]. Examples of OSC formed from alliin and other compounds from garlic are shown in Figure 6-9.

Members of both groups are known as chemopreventive since they interfere with the process. Proposed mechanism explaining this chemopreventive activity include inhibition of activation enzymes (phase I), induction of detoxifying enzymes (phase II, mainly inducible glutathione *S*-transferase), scavenging of the ultimate electrophilic carcinogenic species by the sulphur atom [reviewed in 15], blockage of *N*-nitroso compounds formation (OSC form nitrosothiols from nitrite, one of the substrates for

endogenous formation of *N*-nitroso compounds), enhancement of DNA repair system, reduction of cell proliferation, and induction of apoptosis [17]. Some OSC, mainly oil-soluble ones, are also effective antimicrobial agents [reviewed in 17] and potentially inhibit formation of carcinogens by endogenous microflora. Under prolonged treatment, cells are suggested to adapt to these compounds by changing the rate of absorption or altering metabolic activity [reviewed in 16].

Oil-soluble OSC have higher antiproliferative effect than water-soluble ones, inhibit DNA methylation, important factor in gene regulation, increase histone acetylation and inhibit histone deacetylation [reviewed in 17].

In general, anticancer properties of OSC depend on the number of sulphur atoms in molecule. Thus, diallyl trisulphide is more effective than diallyl disulphide, etc.

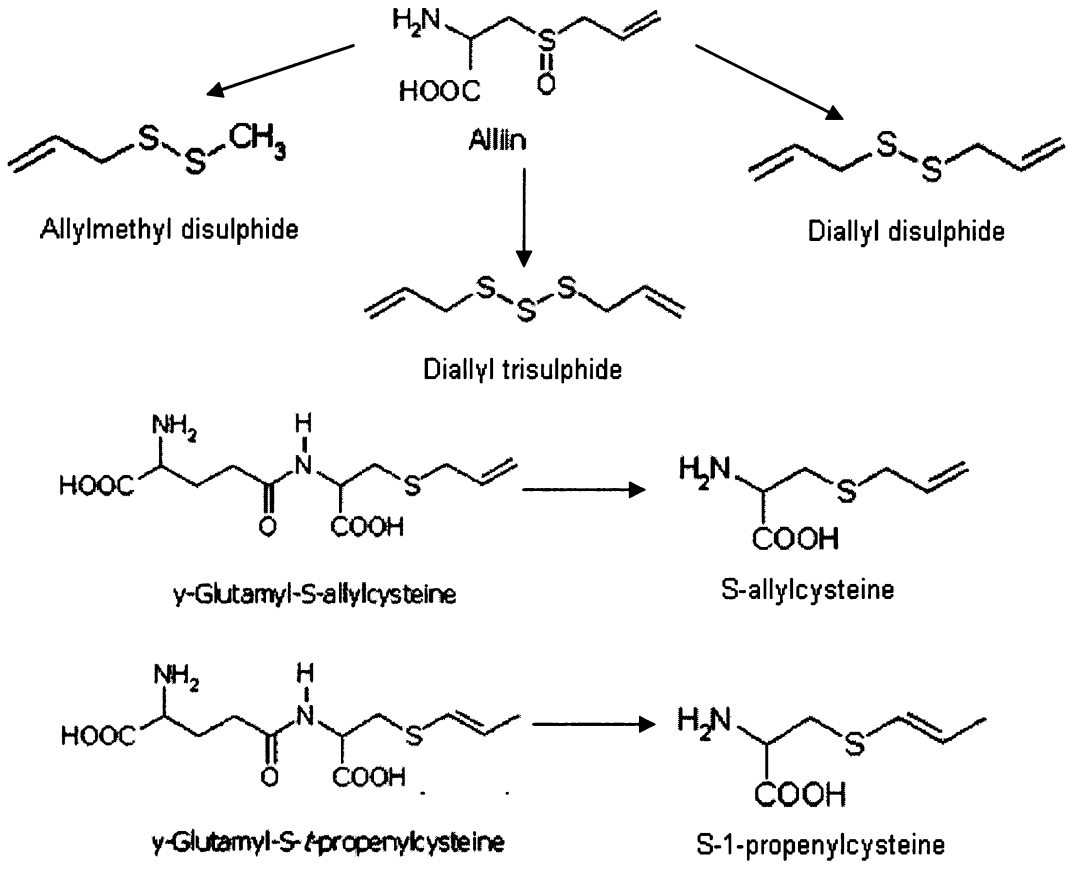


Figure 6-9 Examples of compounds formed in metabolism of alliin and other compounds from garlic

Diallyl sulphide

Diallyl sulphide (DAS) is exclusively present in garlic, and can undergo extensive oxidation on few positions of the molecule. One of them, oxidation of sulphur atom, is mediated by CYPs producing diallyl sulphoxide (DASO) and diallyl sulphone ($DASO_2$)

sequentially [18]. The particular isoform of CYP involved in this metabolism is CYP2E1. The last step, oxidation of terminal double bonds of $DASO_2$ is leading to autocatalytic destruction of this enzyme. First two metabolites are competitive inhibitors of CYP2E1 but not suicidal inhibitors [18]. Because of these inhibitory effects, DAS acts against cancer caused by carcinogens activated by CYP2E1, e.g. *N*-nitroso compounds [95].

DAS has potential to inhibit exogenous formation of other carcinogens in food during cooking, especially HCAs, such as IQ [reviewed in 15].

Modulation of cytochrome P450 activity by organosulphur compounds

Influence of OSCs from garlic on CYP1A family is inconsistent. OSCs inhibited the formation of tumours in animals treated with various carcinogens activated by CYP1A1 and CYP1A2 [16]. On other hand, DAS have been referred to have almost no effect on CYP1A family protein levels [19]. Effect of OSC (natural or synthetic analogues) on CYP1A family levels seems to be time dependent.

Davenport and Wargovich [16] found that diallyl sulphide increases the level of CYP1A1 and CYP1A2 protein in rat liver (Figure 6-10). The level of induction observed was dose and time-dependent but even one dose of DAS (200 mg/kg) increased the protein level of CYP1A1 and CYP1A2 by 684% and 282%, respectively (while the level of mRNA for CYP1A2 was unaffected). The long term study (at maximum 8 weeks, daily dose 200 mg/kg) even increased the protein level of CYP1A subfamily. The increase of CYP1A1 protein level was the most significant, about 600%. Another tested compound, allyl methyl sulphide, which increased protein level of CYP1A2 by 70% when only one dose administered, showed no more increase after 4 weeks of repeated administration, but after 8 weeks CYP1A1 level was enormously enhanced by about 1600% (Figure 6-11). An unexpected effect of DAS on rat liver was found when 200 mg/kg was chronically administrated for 8 weeks. The liver was enlarged and the ratio of liver weight to rat weight was markedly increased, indicating potential liver toxicity. When lower doses of DAS were administrated (50 and 100 mg/kg) chronically for 8 weeks, no liver changes were observed. But interestingly the CYP1A levels were increased only when 50 mg/kg was administrated, the higher dose had no effect (Figure 6-11).

All tested oil-soluble OSC also significantly decreased the CYP2E1 levels and increased CYP3A2 levels either when single dose or chronic doses were administrated [16]. Proposed mechanism for CYP2E1 inhibition is an autocatalytic destruction of this enzyme by DAS and probably by other OSC [18]. DAS is also phenobarbital-type inducer

and extensively increases the protein level of CYP2B family and significantly increases protein level of CYP3A family [19].

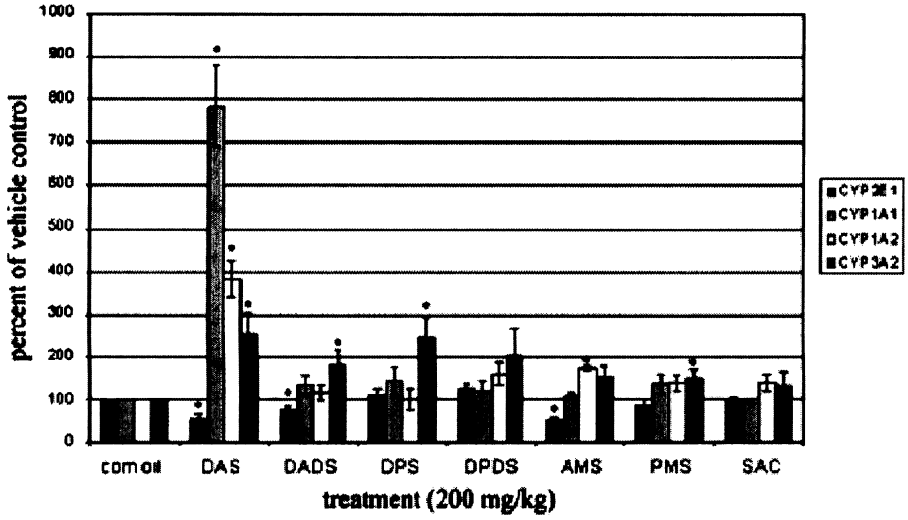


Figure 6-10 Effect of OSCs on hepatic CYP2E1, CYP1A1/2 and CYP3A2 protein levels of male F344 rats, densitometric analysis of Western blot (DAS = diallyl sulphide, DADS = diallyl disulphide, DPS = dipropyl sulphide, DPDS = dipropyl disulphide, AMS = allyl methyl sulphide, PMS = propyl methyl sulphide, SAC = *S*-allylcysteine) (adapted from [16])

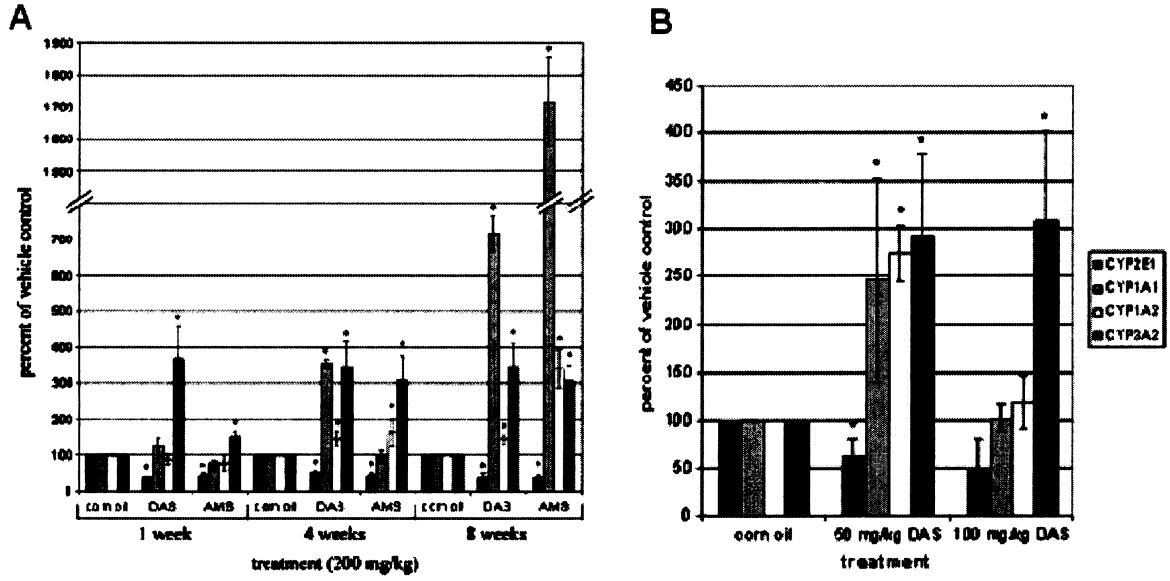


Figure 6-11 Effect of chronic dosing A, of 200 mg/kg DAS and AMS, B, of 50 and 100 mg/kg DAS on hepatic CYP2E1, CYP1A1/2 and CYP3A2 protein levels, densitometric analysis of Western blot (adapted from [16])

6.2.5 Carotenoids

Carotenoids (e.g. β-carotene, lycopene, lutein, bixin, canthaxanthin, astaxanthin), one of the most important group of retinoids, are fat-soluble pigments present in many kinds of fruit and vegetable. They are extensively studied as chemopreventive compounds. Examples of carotenoids are shown in Figure 6-12.

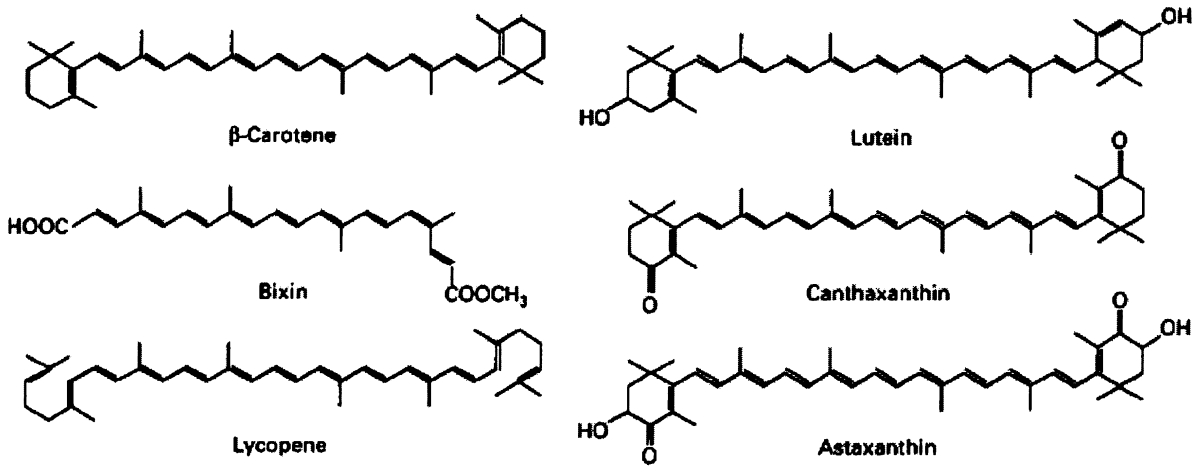


Figure 6-12 Examples of carotenoids (adapted and modified from [46])

Some carotenoids are converted in organism to vitamin A. β-Carotene is a major source of this vitamin for human in natural diet. Many studies have shown beneficial effects of carotenoids reducing cancer risk, partly because of their ability to scavenge reactive oxygen species. β-Carotene also exerts antioxidant properties, ability to influence immune system and enhance gap junction intracellular communication [46]. β-carotene has shown almost no effect on HCA mutagenicity, although better results were seen against mutagenicity of AFB_1, BaP [74]. On the other hand there are also some other studies that demonstrated the increase of cancer risk in smokers who were chronically given high doses of β-carotene [reviewed in 47].

Carotenoids modulate phase I and phase II enzymes, and Jewell et al. [46] determined the effect of some carotenoids on CYPs and GST. The highest induction effect on hepatic CYP activity was seen when canthaxanthin (CX) and astaxanthin (AX) were administrated. When CX was given to rats daily for 16 days, CYP1A1, CYP1A2 and CYP2B1/2 hepatic activities increased 44, 34 and 15 fold respectively. Administration of AX increased these levels 56, 35 and 19 fold. Bixin did not induce CYP activity so intensively, only 6.2, 4.0 and 5.5 fold, respectively. Other tested compounds, β-carotene, lycopene and lutein did not change the activity of these enzymes in liver microsomes.

6.2.6 Curcumin

Curcumin (Figure 6-13), major component of turmeric spice, is present in the root of *Curcuma longa L.*, which exerts anti-oxidative, anti-inflammatory and anti-septic activities. Curcumin interferes with many steps of carcinogenesis, inhibits tumour formation and promotion (cancer initiation, promotion and/or progression is decreased or blocked by this compound) and has ability to induce apoptosis of cancer cells while healthy cells are not affected [reviewed in 36, 49].

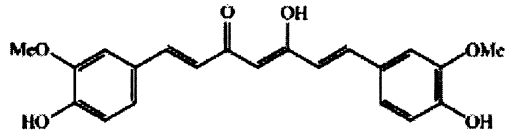
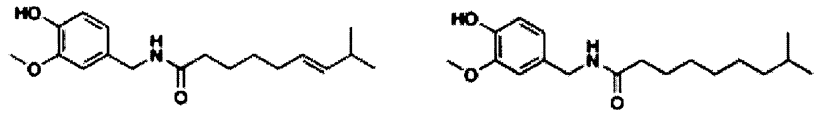


Figure 6-13 Chemical structure of curcumin (diferuloylmethane)

Curcumin and its derivatives (demethoxycurcumin and bis-demethoxycurcumin) inhibit CYP1A1/2 activities *in vitro* in rat liver microsomes at lower concentrations than isothiocyanates and at comparable concentrations inhibit CYP2B1. Because of this, curcumin and its derivatives significantly inhibit activation of BaP [37,75] and decrease the number of BaP and DMBA-derived DNA adducts [75].

6.2.7 Capsaicinoids

Capsaicin (8-methyl-*N*-methyl-6-noneamide) is the main capsaicinoid present in a variety of *capsicum* fruits, such as chillies and peppers. Second most abundant capsaicinoid is dihydrocapsaicin. These two compounds (structure is shown in Figure 6-14) are also about twice as hot as the minor capsaicinoids: nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin.



capsaicin dihydrocapsaicin

Figure 6-14 Chemical structures of capsaicin and dihydrocapsaicin

Capsaicin has been suggested to have chemopreventive effects through modulation of carcinogen metabolisms (e.g. BaP), and interactions with target cell DNA. Pre-treatment of rats with capsaicin for 3 consecutive days resulted in enhancement of activities of pulmonary anti-oxidant enzymes, such as superoxid dismutase, catalase and peroxidase, while long-term treatment caused inhibition of latter two enzymes. Capsaicinoids exert

inhibitory effects on liver microsomal CYP2E1, CYP1A1/2 and CYP2B1/2 activities [reviewed in 59].

On other hand, capsaicin is metabolized by CYP2E1 into reactive species that are capable of covalent binding to active site of enzyme and other macromolecules, such as DNA and proteins. These covalent modifications lead to toxicity, including mutagenesis, carcinogenesis, and suicidal inhibition of CYP enzymes [59].

6.2.8 Alkylbenzenes

Alkylbenzenes, such as *trans*-anethole and eugenol (Figure 6-15), naturally occur in food flavourings and spices. *trans*-Anethole is the major volatile component in bitter fennel and anise, and is present in at least other 20 spices. Eugenol is the main constituent in clove oil and is also in spices, such as cinnamon, basil and nutmeg.

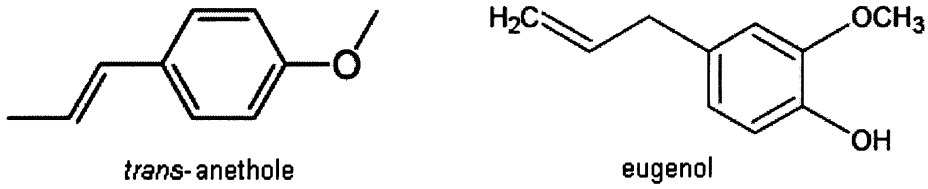


Figure 6-15 Chemical structures of *trans*-anethole and eugenol

Some studies suggested these compounds as chemopreventive because of their potential to induce phase II enzymes. Abraham [63] pre-treated mice with eugenol and *trans*-anethole and sequentially co-administrated carcinogens, such as cyclophosphamide, ethyl methane sulphonate, urethane, procarbazine and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and measured the frequencies of micronucleated polychromatic erythrocytes in bone marrow. Both these agents decreased the genotoxicity of urethane, procarbazine, cyclophosphamide and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and *trans*-anethole was also effective against ethyl methane sulphonate mutagenicity.

On the other hand alkylbenzenes, such as methyleugenol, estragole and safrol, have shown mutagenic and cytotoxic properties themselves after activation by CYP and sulfotransferases forming ultimate electrophiles [57].

6.2.9 Caffeine

Caffeine (sometimes called guaranine when found in guarana, mateine when found in mate, and theine when found in tea) is a xanthine alkaloid found in the leaves and beans of the coffee tree, in tea, yerba mate, guarana berries, and in small quantities in cocoa, the kola nut and the Yaupon holly. Structure of caffeine is shown in Figure 6-16.

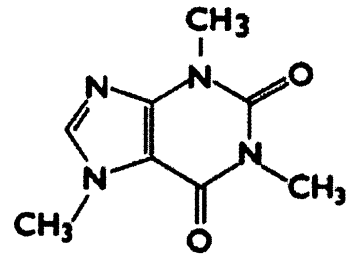


Figure 6-16 Chemical structure of caffeine

Effect of caffeine is usually not studied as a particular compound, but is present in some plant extracts used. Few studies are focused on the influence of caffeine on PhIP metabolism and PhIP-induced carcinogenesis. But results from these experiments are inconsistent. Caffeine decreased the mutagenicity of PhIP in Ames test and micronucleus assay, and simultaneous administration of PhIP and caffeine slightly decreased mammary gland tumour formation in female F344 rats. Interestingly, the incidence of colon carcinomas increased [76]. Unexpectedly, level of CYP1A2 mRNA was higher when PhIP and caffeine were co-administrated than administrated alone [76]. No modifying effect of caffeine on 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) induced hepatocarcinogenesis was evident although CYP1A2 mRNA level was significantly increased [77].

6.2.10 Kahweol and cafestol

Kahweol and cafestol (Figure 6-17) are two diterpens present in coffee beans and in unfiltered or boiled coffee drinks. Both of these agents significantly elevate the cholesterol levels but they are also known to have some beneficial effects. They possess a strong chemopreventive potential, protect cells against mutagenesis and carcinogenesis in animal models [reviewed in 36]. They prevent the formation of DNA adducts of several genotoxic carcinogens (e.g. AFB_1, PhIP), inhibit the formation and/or stimulate detoxification of electrophilic or oxidative intermediates [38].

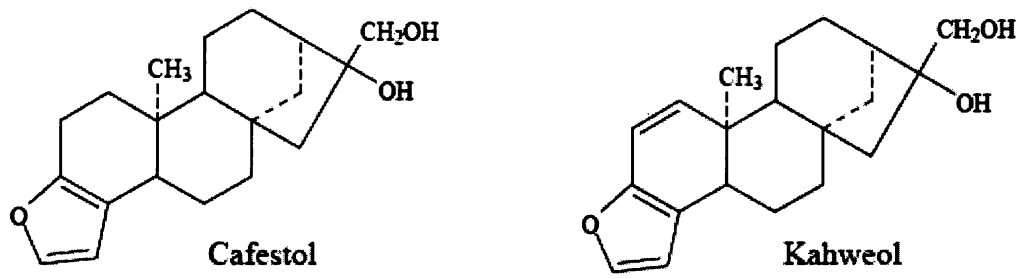


Figure 6-17 Chemical structures of cafestol and kahweol

Majer et al. [39] observed the inhibition of NDMA-induced genotoxicity by mixture of these two compounds. In Figure 6-18 are shown data from Cavin et al. study [38] showing potential of these compounds to inhibit AFB_1-induced carcinogenesis.

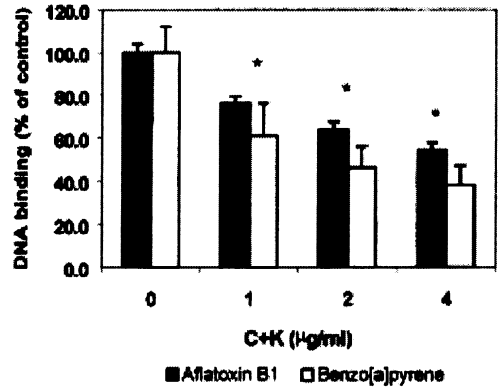


Figure 6-18 Dose-dependent effect of cafestol (C) and kahweol (K) on the formation of AFB_1 and BaP induced adducts in vitro (adapted from [38])

Kahweol and cafestol are known to inhibit phase I enzymes (primarily CYPs), and induce phase II enzymes (GST). Diet containing kahweol and cafestol decreased significantly CYP3A2 mRNA and protein levels, and inhibited CYP3C11 in rats. Both these effect were confirmed on primary hepatocyte cultures [reviewed in 38]. In another study, Majer et al. [39] tested inhibitory effect of these compounds on other CYPs involved in activation of AFB_1 and PhIP. Interestingly, there was no detectable difference in the activity of CYP1A1 in human derived hepatoma (HepG2) cells.

6.2.11 Herbal extracts and other compounds

Last ten chapters were focused on particular compounds and their effects on carcinogenesis. But indispensable part of these studies investigates the chemopreventive potential of plant or herbal extracts (complex mixtures), not a single defined component or compound. Influence of these mixtures and some of their particular compounds on carcinogenesis will be discussed in this chapter.

Tea (Camellia sinensis)

Tea is a common beverage made by processing the leaves or buds of the tea bush *Camellia sinensis*, containing many antioxidant phenolic constituents (mentioned above in Chapter 6.2.1). A typical green tea contains catechins (e.g. epicatechin, epigallocatechin, and the major component epicatechin-3-gallate), flavonols (e.g. quercetin and myricetin), caffeine, and theobromine. Black tea undergoes fermentation, during which high amount of catechins is converted into dimeric theaflavins and polymeric thearubigins.

Many studies with animal models have been done to determine anti-tumour and cancer preventive activities of tea. Most of these studies confirmed, that tea (black or green) provides beneficial effects against carcinogenesis [30]. Catterall et al. [31] investigated the influence of black tea theaflavins and theafulvins on CYP levels and activities in rat liver and intestine. Interestingly, hepatic CYP activities were unaffected but in small intestine activities of CYP1A and CYP2E subfamilies were decreased. In another study [reviewed in 32] black and green tea solutions given to male F344 rats for 6 weeks increased hepatic CYP levels (1A1, 1A2, 2B1). Black and green tea solutions most extensively increased CYP1A2 level by 623% or 540%, respectively.

The effect of caffeine in tea extracts was also determined. Caffeine even decreased mutagenicity of PhIP on *Salmonella* strains, when effects of normal and decaffeinated tea were compared [reviewed in 32]. Similar study, made by Chen et al. [33], observed that caffeine induces CYP1A2 activity in male rat F344 liver microsomes. Black and green tea induced CYP1A2 while decaffeinated tea did not induce CYP1A2 activities. Moreover, there was found correlation between caffeine plasma levels and induction of CYP1A2, while CYP activity did not correlated with tea phenolic compound levels.

Rosemary extract

Extracts from *Rosmarinus officinalis L.* may be prepared under various conditions and because of this every extract contains various compounds in diverse amounts. Polyphenolic fractions of rosemary extract exhibit strong antioxidant properties. Carnosol and carnosic acid (Figure 6-19), two phenolic diterpens that are highly abundant, are thought to be the most potent antioxidants of these extracts. These two compounds together with other components inhibit initiation and tumour promotion stages of carcinogenesis in mouse and rat models [reviewed in 40].

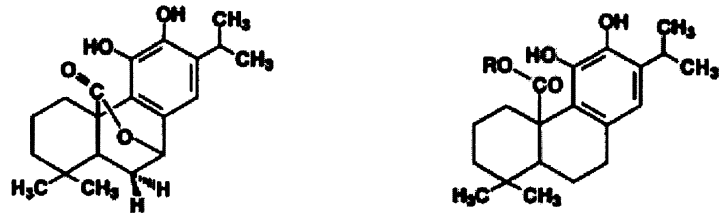


Figure 6-19 Chemical structures of carnosol and carnosic acid

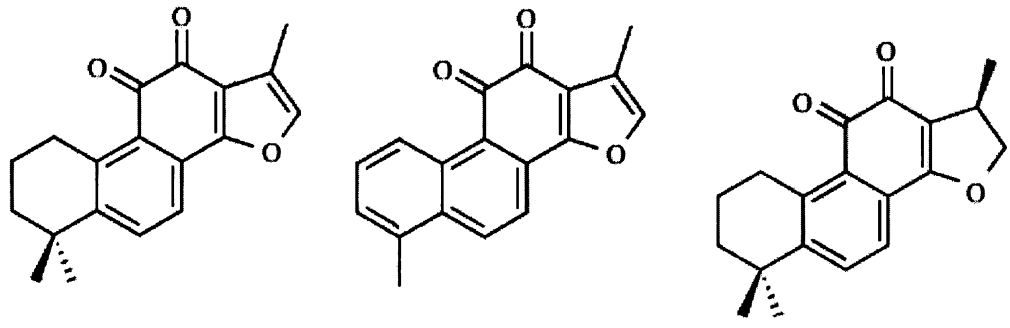
Anticarcinogenic properties of rosemary extract consists in modulation of phase I (inhibition) and phase II enzymes (induction). Offord et al. [40] has confirmed the

inhibition of CYP1A1/2 and CYP3A4 *in vitro* using human liver and bronchial cell models. In Debersac et al. study [41], essential oil and water-soluble extract from rosemary leaves induced CYP1A1/2 and CYP2B1/2 hepatic activity in rats. After the treatment with essential oil, protein levels of CYP1A1/2 were not detectable but CYP2B1 level was significantly increased.

Salvia miltiorrhiza and Ginkgo biloba

Tanshinone is a major active ingredient in *Salvia miltiorrhiza* extract (also named Dan Shen), which is widely used in traditional Chinese medicine, similarly to *Ginkgo biloba,* for treatment of cerebrovascular or cardiovascular diseases. Tanshinone exerts antioxidant and anti-inflammatory properties, and anti-tumour potential [42], in high, cytotoxic concentrations induce apoptosis and in lower concentration restores cell differentiation [97]. Structure of tanshinone and its derivatives that are present in Dan Shen are shown in Figure 6-20.

Tanshinone is also known inducer of many CYP isoforms. For example when a dose 100 mg/kg was given daily to rat for 10 days, activity of CYP1A2, CYP1A1 and CYP2B1 in rat liver increased significantly, 7.6 fold, 7.1 fold and 2.1 fold, respectively. Activity of CYP2E1 decreased significantly, 1.9 fold, and activity of CYP3A did not change markedly [42].



Tanshinone IIA Tanshinone I Cryptotanshinone

Figure 6-20 Chemical structures of tanshinone and its derivatives

Ginkgo biloba contains terpenoids and about 30 different flavonoids [reviewed in 42], and is used for the treatment of cerebral insufficiency and peripheral vascular diseases [reviewed in 43]. Because of high flavonoid content, *Ginkgo biloba* extracts exert antioxidant properties, too.

This extract also modulates CYP activity. Yang et al. [42] determined rat hepatic CYP activities after 10 day treatment with two doses of *Ginkgo biloba* extract, 100 mg/kg and 200 mg/kg. The higher dose increased activity of CYP1A2, CYP1A1 and CYP2B1,

2.3 fold, 9.4 fold and 8.3 fold, respectively. Activity of CYP3A and CYP2E1 did not increased significantly, 1.6 and 1.4 fold, respectively. When lower doses were administrated, activity of all hepatic cytochromes did not change markedly.

In another study, of Sugiyama et al. [43], rats were fed with 0.5% *Ginkgo biloba* extract for one week. The total hepatic CYP protein content increased more than 4 fold, and activity of some CYP isoforms increased significantly. Activity of CYP1A1, CYP2B, CYP2C9, CYP2E1 and CYP3A4 increased 2.7, 27, 4.6, 2.7 and 5.4 fold, respectively, but activity of CYP1A2 did not changed.

Broccoli (Brassica oleracea)

Broccoli contains many different active compounds that modulate carcinogenic process. They can act as inducers of phase I and phase II enzymes [44]. When 500g of fresh broccoli was given daily to 18 volunteers for 12 days after 6 day period with low induction diet, then activity of CYP1A2, CYP2E1 and GST were measured. No effect was observed on CYP2E1 and GST activity, but CYP1A2 activity increased by 19% [reviewed in 44].

Some compounds present in *Cruciferae* plant (*tert*-butylhydroquinone, 3,3'-diindolylmethane, ascorbigen, indole-3-carbinol, sulforaphane, benzyl isothiocyanate and phenetyl isothiocyanate) were tested by Bonnesen et al. [70] for their ability to modulate CYP activities, protein and mRNA levels in human colon cell lines, LS-174, Caco-2, and HCEC. Interestingly, high differences were found among these cell lines. Results from this measurement are shown in Table 6-4. Treatment with some of these phytochemicals induced CYP1A1 activity in LS-174 and Caco-2 cell lines, but not in HCEC. The inducibility of this enzyme was highest in LS-174 cell lines, but not all phytochemicals, that induced activity in Caco-2 cells, were able to induce CYP1A1 in this cell line. These results were confirmed using Western blotting method and RT-PCR method.

Table 6-3 Differences in LS-174, Caco-2, and HCEC induction of CYP1A1 activity (β-NF = β-naphtoflavone, *t*-3HQ = *tert*-butylhydroquinone, DIM = 3,3'-diindolylmethane, ASG = ascorbigen, I3C = indole-3-carbinol, ICZ = indolo[3,2-*b*]carbazole, SUL = sulforaphane, BITC = benzyl isothiocyanate and PEITC = phenetyl isothiocyanate)

	EROD activity (μmol resorufin formed/min/mg protein) on treatment with									
Cell line	Control	β-NF	t-BHQ	DIM	ASG	I3C	ICZ	SUL	BITC	PEITC
LS-174	<5	910±103	<5	885±55	705±45	560±65	1040±150	<5	<5	<5
Caco-2	355±55	1325±385	470±95	1210±263	690±280	830±160	1255±350	380±70	355±45	400±30
HCEC	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40

Adapted from [70]

Glucoraphanine, a precursor of sulforaphane present in broccoli and *Brassicaceae*, was confirmed as an inducer of phase I and phase II enzymes by Perocco et al. [45]. This compound was also capable of generating different radical reactive species. In this study 120 mg/kg and 240 mg/kg dose of glucoraphanine were given daily for 4 days to rats and activity of some CYP isoforms was measured. Activities of CYP1A1, CYP3A1/2, CYP2E1, CYP1A2, CYP2B1/2 and CYP2C11 increased markedly (from 1.1 to 5.7 fold), while activity of phase II enzymes, for example GST, did not increase so significantly (only 1.6 fold in maximum).

Liquorice root extract

Liquorice root extract (from *Glycyrrhiza glabra L.*) contains many constituents, such as glycyrrhizin (G), saponin-like glycoside, flavonoid glycosides (e.g. rhamnoliquiritin, rhamnoisoliquiritin, isoliquiritoside, liquiritin, isoliquiritin and liquiritisolide), coumarin derivatives, mannitol, glucose and many others. This extract and especially glycyrrhizin (Figure 6-21) was observed to have anti-arthritic, anti-allergic, anti-estrogenic, anti-viral, anti-hepatotoxic, anti-cholinergic, anti-inflammatory, anti-leukaemogenic and anticarcinogenic properties. The latter is usually associated with its ability to induce a variety of enzymes.

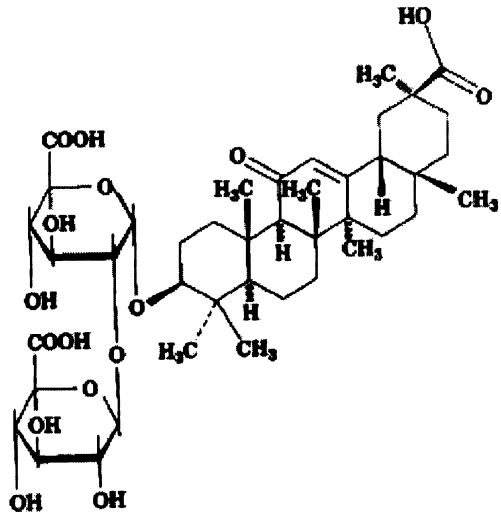


Figure 6-21 Chemical structure of glycyrrhizin

Paolini et al. [64] investigated effect of G on enzymes involved in carcinogen metabolism and activation. Single dose of either G or liquorice extract did not affect CYP activities but administration for four consecutive days (four doses) enhanced CYP1A2 and CYP3A activities.

6.3 Synthetic chemopreventive compounds

Nonsteroidal anti-inflammatory drugs

Some nonsteroidal anti-inflammatory drugs (NSAIDs) exhibit chemopreventive properties. Aspirin and its derivatives, such as celecoxib, piroxicam, sulindac with its metabolites, and lovastatin, are selective inhibitors of cyclooxygenases (COX). COX enzymes, especially inducible COX-2 activity is usually induced in early stages intestinal tumours, resulting in inhibited apoptosis of cells and carcinogenesis. Treatment with piroxicam decreased the incidence of intestinal tumour multiplicity by 68% when 200ppm doses were administrated daily for either 100 or 200 days to mice. On the other hand, in other studies, this dose of piroxicam resulted in intestinal ulceration in more than 90% of treated mice. In clinical test on human, when 20 mg of this agent were chronically taken, about 20% of individuals experienced significant gastrointestinal side effects, such as dyspepsia and intestine perforation [49].

Selective estrogen receptor modulators

Estrogen antagonist can be potentially used in high risk breast cancer women to prevent developing of this type of cancer. Agents used are selective estrogen receptor modulators (SERMs), such as tamoxifen, raloxifene, retinoids, RXR-selective targretin, RAR-selective fenretinide and its derivatives, and fenretinide derivatives [49].

Steroids

From steroids, dehydroepiandosterone (DHEA) has been found to inhibit generation of NADPH, which is necessary for activation reactions catalyzed by mixed function oxidases [49].

Administration of all of these synthetic chemopreventive agents was beneficial in animal models, decreasing the incidence of various types of cancer, but some of them were also found to have negative side effects.

Sulphur containing compounds

Oltipraz (5-(2-pyrazinyl)-4-methyl-1,2-dithiolthione), synthetic chemopreventive sulphur containing compound, is similar to dithiolthiones found in cruciferous vegetable. Oltipraz is known inducer of detoxification enzymes (e.g. GST) as well as inhibitor of some CYPs involved in activation of carcinogens (e.g. CYP3A4 and CYP1A2). This compound is now in phase II of clinical studies [49,61].

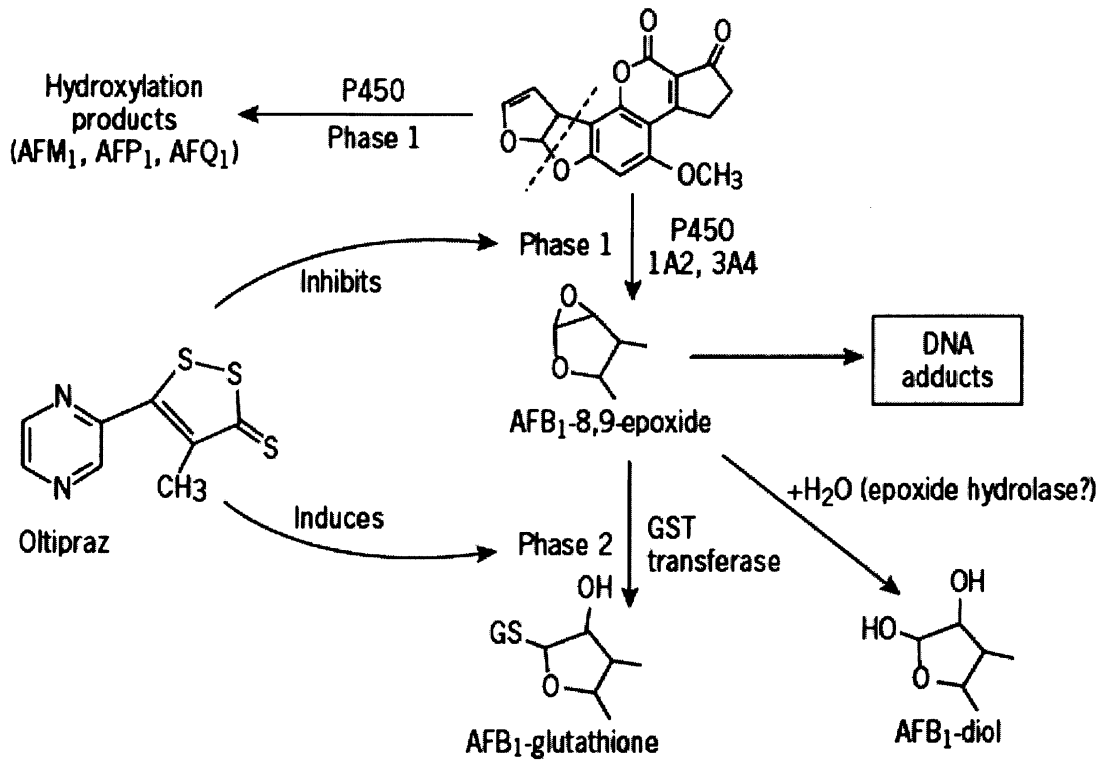


Figure 6-22 Effect of oltipraz on aflatoxin B_1 (AFB_1) induced carcinogenesis; P450 = cytochrome P450, GST transferase = glutathione *S*-transferase (adapted from [3])

Amilorides

Amiloride is normally used as a diuretic drug. Amiloride and its derivatives are known to act as inhibitors of the Na^+/H^+ exchanger, have been reported to inhibit tumour growth both *in vitro* and *in vivo*, and to posses chemopreventive effects against *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in rodents. 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA), one of amiloride derivatives, was investigated by Sparfel et al. [65] in connection with its ability to modulate CYP activity. They observed competitive inhibition of EIPA with CYP1A1 substrates (e.g. PAHs), and also inhibition of CYP1A2 and CYP1B1 activity, while the levels of CYP mRNA were not affected.

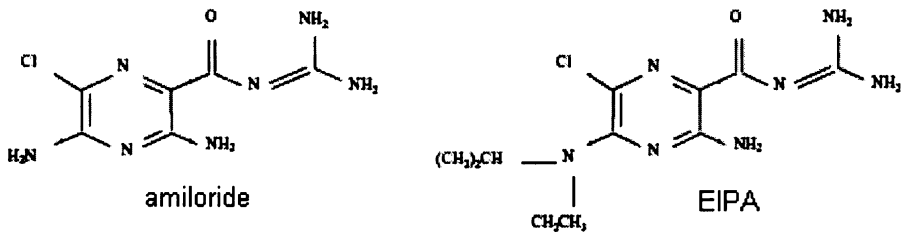


Figure 6-23 Chemical structures of amiloride and 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA) (adapted and modified from [65])

7 Conclusions and future remarks

Diet components are thought to cause about one third of cancer incidents. This might be the reason why recently, alternative medicine is getting more popular in Western countries. An enormous amount of dietary supplements and functional food is sold every year. People tend to take these supplements to prevent various diseases and reduce cancer risk. The significant increase of their popularity is documented in Figure 7-1. In 2003 the global market value of dietary supplements in whole world was 56 billion dollars, and Europe took about one third (19 billion dollars).

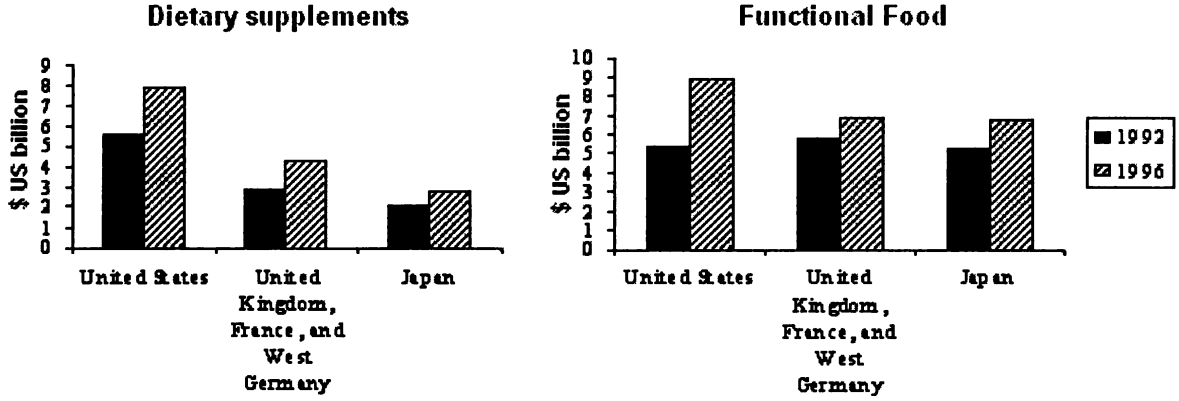


Figure 7-1 Global market value of nutraceuticals in 1992 and 1996 (data used from [50])

Traditional medicine uses various herbs and plants that contain diverse chemical compounds. These mixtures modulate many enzymes and the resulting effect is balanced and usually beneficial – some of them induce particular enzymes while others inhibit them. Even the extracts are of different composition than herbs and plants themselves, depending on conditions used for extraction. Moreover, many commercial dietary supplements based on these herbal extracts have different composition. These extracts and supplements vary in modulation effects on enzyme activities, and might provide negative effects.

People think that these "natural" preparations are in all cases healthful and safe, and that they cannot hurt them. But when they are consumed in high enough amounts, for a long enough time, or in combination with certain other substances, all chemicals can be toxic, including nutrients, plant components, and other biologically active ingredients.

Recently, one of the most important questions addresses beneficial effects of dietary supplementations. Chemopreventive compounds affect enzymes in whole body complexly but they are usually tested for their anticancer activity under artificial conditions (*in vitro* experiments), and the results obtained in these simplified systems are extrapolated

to *in vivo* conditions. Under complex conditions of the whole body, the declared beneficial activity of such compounds is then hardly proved. Moreover, some recent studies have shown that some used chemopreventive compounds can in some cases even enhance the cancer risk (e.g. β-carotene [47]) or exert mutagenic properties (e.g. quercetin [29,57]). Because of this, dietary supplements containing these compounds should be taken with caution.

One of high risk factors, enhancing the cancer disease development, is induction of enzymes (mainly cytochromes P450 – Table 7-1) that are involved in activation of food carcinogens. The beneficial inhibitory effect of chemopreventive compounds is detected only if they are taken simultaneously with carcinogens. Moreover, the dose of chemopreventive compound must be by many times higher than the carcinogen intake (dose). When the carcinogen is ingested much later than the chemopreventive compound, the concentration of this compound is too low to accomplish the desired chemopreventive effect, while the induction of cytochrome(s) P450 might reach the maximum and the carcinogen activation can be enhanced.

Table 7-1 Human cytochromes P450 involved in activation of food carcinogens

CYP isoform	activated food carcinogens
1A1	polycyclic aromatic hydrocarbons (e.g. BaP) PhIP
1A2	heterocyclic amines (e.g. Glu-P-1, Glu-P-2, Trp-P-2, IQ, MeIQx) aflatoxins
2A6	*N*-nitroso compounds (e.g. NDMA, NDEA, NDPA, NDBA) aflatoxins
2B1	*N*-nitroso compounds (e.g. NDPA, NDBA, NBPA)
2E1	*N*-nitroso compounds (e.g. NDMA, NDEA, NPYR, NMBA, NPRO)
3A4	aflatoxins (e.g. B_1, G_1) sterigmatocystin dihydro-polycyclic aromatic hydrocarbons (e.g. dihydro BaP)

The list of plant chemopreventive compounds, extracts and mixtures that are discussed above (in Chapter 6) is shown with their modulation effects on particular CYP isoforms in Table 7-2.

Table 7-2 Chemopreventive inducers and inhibitors of cytochrome P450, for more data about flavonoids and phenolic compounds, see Table 6-2

CYP isoform	inducer	inhibitor
1A1	indole-3-carbinol [21] diallyl suphide [16] allylmethyl sulphide [16] canthaxanthin [46] astaxanthin [46] tea [32] tanshinone [42] *Ginkgo biloba* extract [42,43] essential oil rosemary extract [41] glucosinolates (*brassica* veg.) glucoraphanine [45] flavonoids and phenolic compounds	resveratrol [24,25,48] curcumin [75] isothiocyanates [23,48] capsaicin [59] theafulvins [31] theaflavins [31] rosemary extract [40] flavonoids and phenolic compounds
1A2	indole-3-carbinol [21] diallyl suphide [16] canthaxanthin [46] astaxanthin [46] caffeine [33] tea [32] tanshinone [42] *Ginkgo biloba* extract [42] glycyrrhizin [64] essential oil rosemary extract [41] broccoli [44] and cruciferous veg. glucoraphanine [45] flavonoids and phenolic compounds	resveratrol [24,25,48] curcumin [75] isothiocyanates [23,48] capsaicin [59] theafulvins [31] theaflavins [31] rosemary extract [40] flavonoids and phenolic compounds
2B1/2	indole-3-carbinol [21] diallyl sulphide [16] canthaxanthin [46] astaxanthin [46] essential oil rosemary extract [41] tea [32] tanshinone [42] *Ginkgo biloba* extract [42,43] glucoraphanine [45] flavonoids and phenolic compounds	resveratrol [24,25,48] curcumin [75] isothiocyanates [23,48] capsaicin [59] flavonoids and phenolic compounds
2E1	*Ginkgo biloba* extract [42,43] glucoraphanine [45]	resveratrol [25] capsaicin [59] diallyl suphide [18] organosulphur compounds [18] isothiocyanates (cruciferous veg.) theafulvins [31] theaflavins [31] tanshinone [42] flavonoids and phenolic compounds
3A4	indole-3-carbinol [21] glycyrrhizin [64] *Ginkgo biloba* extract [43] glucoraphanine [45] flavonoids and phenolic compounds	resveratrol [25] kahweol and cafestol [38,39] rosemary extract [40] flavonoids and phenolic compounds

As an example of negative impact could be mentioned co-administration of benzo[a]pyren (BaP) and flavonoids: When BaP is ingested simultaneously with flavonoids (e.g. wogonin), the DNA-adducts formation is reduced [37]. In the contrary, when flavonoids and BaP are administrated sequentially, the particular isoform of cytochrome P450 (CYP1A1) that is involved in activation of BaP may be induced and the activation may be enhanced.

Moreover, none of these chemopreventive compounds is universal. Each compound acts against special type of carcinogens, for example by scavenging its precursors or reactive metabolites. But this particular compound also influences metabolism of other carcinogens. Result might be the decrease of one carcinogen activation while others activation is enhanced.

However, understanding of these possibly negative influences is of high importance, nowadays, there is only a negligible number of studies that are focused on sequential administration of plant chemopreventive compounds and carcinogens. Further research in this area is necessary. Better knowledge of this problem could prevent developing of some cancer incidences.

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