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Antiproliferační účinky produktů katabolické dráhy hemu

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty Iniverzity Karlovy v Praze.

Abstrakt

Předkládaná práce se zabývá katabolickou dráhou hemu s hlavním zaměřením na žlučové pigmenty.

Ze začátku jsme se zaměřili na ne příliš známé aspekty metabolismu bilirubinu ve střevních bakteriích. Dále jsme se zabývali neurotoxickými účinky bilirubinu, které jsme studovali na zvířecím modelu závažných nekonjugovaných hyperbilirubinemií, tj. na hyperbilirubinemických Gunnových potkanech. Zkoumali jsme distribuci a odbourávání bilirubinu v mozku za patologických podmínek, jako jsou např. Criglerův-Najjarův syndrom či novorozenecká žloutenka. Naším cílem bylo také možné zlepšení léčby nekonjugovaných hyperbilirubinemií, které spočívá v kombinaci fototerapie a podávání lidského albuminu. Náš následný výzkum byl zaměřen na protinádorový vliv dalšího významného produktu katabolismu hemu- oxidu uhelnatého. Na základě zjištění, že mechanismy neurotoxického působení bilirubinu mohou být shodné s jeho protinádorovými a zdraví prospěšnými účinky, studovali jsme bilirubin a bilirubinu podobné kyanobakterilální/rostlinné tetrapyroly a prokázali jejich antioxidační a protinádorové působení.

Záměrem této práce bylo tedy objasnit některé aspekty katabolismu hemu se zaměřením na antiproliferační vliv jeho produktů.

Abstract

In the center of the presented work is heme catabolism with the main focus on bile pigments.

At the beginning not properly known aspects of bilirubin metabolism in intestinal bacteria were studied. Further we were concerned with neurotoxic effects of bilirubin. The animal model of severe hyperbilirubinemia- jaundiced Gunn rats, was used. We studied bilirubin distribution in the brain tissue and its degradation during pathological conditions, such as newborn jaundice or Crigler-Najjar syndrome. Our purpose was as well possible improvement of unconjugated hyperbilirubinemias treatment- combination of phototherapy and human albumin administration. Our next investigation was directed on anticancer impact of other important heme catabolic pathway´s product- carbon monoxide. Based on the finding, that the mechanism of bilirubin neurotoxicity are most probably similar to its anticancer and health protective impact, bilirubin and bilirubin like cyanobacterial/plant tetrapyrroles were investigated and their important antioxidant and anticancer activities were proved.

The aim of this study was thus to clarify some aspects of heme catabolism with respect for antiproliferative properties of its products.

Content

1 Introduction

1.1 Heme catabolic pathway

Heme catabolic pathway is responsible for heme degradation and serves as a source of biologically active molecules, such as carbon monoxide (CO), ferrous ion, biliverdin (BV) subsequently reduced to unconjugated bilirubin (UCB), that have impact on cellular functions [1]. The main source of heme represents hemoglobin from senescent or damaged red blood cells. Other heme sources include myoglobin, as well as heme-containing enzymes.

In the first catabolic and rate-limiting step, the tetrapyrrolic cycle of heme is opened by microsomal enzyme heme oxygenase (HMOX, EC 1.14.99.3). This reaction occurs mainly in the reticuloendothelial system and yields equimolar quantities of CO, ferrous iron and BV. HMOX has been detected in prokaryotic bacteria, plants, fungi, as well as mammals [2, 3]. HMOX is a phylogenetically well conserved protein, which indicates its physiological importance. The HMOX is essential for human life, as its deficiency results in death [4, 5].

CO is gaseous molecule that belongs to the endogenously produced signaling transmitters with important biological effects, such as nitric oxide or hydrogen sulfide [6]. Nevertheless, high CO concentration is toxic to humans due to approximately 200 times higher affinity to hemoglobin compared to oxygen and thus causes tissue suffocation [7]. On the other hand, CO has various beneficial effects, such as anti-inflammaory [8], apoptosis-modulating [9], anti-atherogenic [10], anti-proliferative [11] and cytoprotective activities [12, 13].

1.2 Bilirubin - from its formation to urobilinoids

Bilirubin is a product of BV reduction catalyzed by biliverdin reductase (BLVRA, EC 1.3.1.24). The reduction at the central carbon (C10) makes UCB a non-polar molecule with unique biological properties.

UCB was for a long time viewed only as a potentially neurotoxic by-product. Research in recent years shed light on important biological impact of UCB. UCB is considered a major antioxidant in blood and even more, its higher physiological concentration are connected with lower prevalence of various civilization diseases [14-16].

In blood circulation UCB is tightly bound to albumin. Non-bound UCB fraction, approximately 0.01%, is known as free bilirubin (Bf), which is responsible for its biological effects. UCB neurotoxicity may occur when Bf overcomes the aqueous solubility of UCB (which is 70 nM) [17]. Bf activates aryl hydrocarbon receptor (AhR), which may lead to increased transcription of P450 monooxygenases (Cyp), as described in jaundiced Gunn rats [18]. In turn, Cyp oxidize UCB to more polar metabolites easily secreted into bile. Cyp can even participate in UCB elimination like cellular defense against high Bf levels [19]. It was discovered that drugs with higher affinity to UCB binding sites on albumin can displace the pigment and cause severe elevation of Bf. This was described in newborns treated with sulphonamides, who subsequently developed kernicterus in the presence of relatively low plasma bilirubin levels [20]. The importance of Bf for pathophysiology of bilirubin encephalopathy was highlighted by Ahlfors et al. by the observation that

auditory brainstem response screening, a quantifiable method to evaluate the bilirubin-induced neurotoxicity, correlates with Bf rather than with total bilirubin concentration in the blood [21].

Albumin bound UCB is carried into the liver, where it is conjugated with glucuronic acid by bilirubin UDP-glucuronosyl transferase (UGT1A1, EC 2.4.1.17). This conjugation dramatically changes bilirubin properties and makes it a polar substance that is actively secreted into bile by canalicular multidrug resistance-related polypeptide (MRP2), a member of the adenosine triphosphate (ATP)-binding cassette (ABC) family of transporters. Conjugated bilirubin is then transported *via* bile duct system into the digestive tract. Intestinal microflora deconjugates bilirubin releasing UCB and glucuronic acid. UCB undergoes further reduction to non-toxic and more polar substances called urobilinoids. Despite the importance of these process only a few bacterial strains capable of UCB reduction have been described: *Clostridium ramosum*, *Bacteroides fragilis*, *C. difficile* and *C. perfringens* [22]. The production of urobilinoids is highly efficient in adults when compared to infants during the first month of life. Low urobilinoid production in neonates is due to undeveloped intestinal microflora capable of reducing UCB. Detailed intestinal metabolism of bilirubin has not been clarified so far, enzyme(s) responsible for bilirubin reduction are awaiting to be identified.

1.3 Hyperbilirubinemias

Elevated serum bilirubin levels above the physiological range (2-17 μmol/L) are marked as hyperbilirubinemias. Hyperbilirubinemias are usually

classified according to the type of elevated bilirubin as unconjugated (premicrosomal), conjugated (postmicrosomal) or mixed hyperbilirubinemia.

The common cause of unconjugated hyperbilirubinemias is UCB overproduction (such as from hemolysis or ineffective erythropoesis), disordered bilirubin uptake by hepatocytes or its conjugation within the liver cell. The latter is exemplified in subjects with benign hyperbilirubinemia caused by inherited partial deficiency of UGT1A1, known also as Gilbert syndrome (GS). GS occurs in about 3-10% of the population [23-25] and is associated with decreased risk of oxidative stress-mediated diseases including cancer [26]. On contrary, in Crigler-Najjar (CN) syndrome patients characterized by complete (CN syndrome type I) or less severe deficiency (CN syndrome type II) of UGT1A1, severe jaundice appears during the first days of life and persists thereafter. Serum bilirubin can reach extremely high concentrations (340-770 μmol/L), which may result in development of severe neurologic complications such as hearing problems, mental retardation, or even apparent kernicterus [27, 28]. For CN syndrome I patients liver transplantation is considered as efficient treatment or life-long phototherapy resulting in an elimination of water-soluble photoisomers of UCB *via* bile. The efficacy of the phototherapy may decrease gradually with age and patients are at higher risk of sudden brain damage [29].

Physiological neonatal jaundice (UCB up to 340 μmol/L, where the kernicterus seldom occurs [30]), is believed to be a part of natural defense against increased oxidative stress [17, 31] and has multifactorial etiopathogenesis [32]. The major factors include

enhanced degradation of fetal hemoglobin, immature liver conjugation system or slow colonization of neonatal gastrointestinal tract with bilirubin-reducing bacteria [22]. High UCB serum levels above 220 μmol/L occurs in approximately 8-20% full-term newborns [33] and may be due to multiple causes, including neonatal prematurity, hemolysis (such as from Rh incompatibility), glucose-6-phosphate dehydrogenase deficiency, sepsis, or rare CN syndrome. Severe hyperbilirubinemia may lead to development of acute bilirubin encephalopathy. The acute stage is manifested with somnolence, hypotonia, poor sucking reflex [34] and if unrecognized and untreated, it can be followed by an irreversible bilirubin neurologic damage [35] known as kernicterus [36]. In this serious disease, the basal ganglia, specifically globus pallidus, subthalamic nucleus, brainstem nuclei (especially the auditory), oculomotor, vestibular nuclei, cerebellum and colliculi are seriously affected. Severe neonatal jaundice is usually treated by phototherapy, where the UCB levels are decreased by UCB photoisomeration, where the more polar photoisomers can be excreted into bile without conjugation. Phototherapy is generally considered to be a safe treatment. However, some side effects have been described, like interference with infant-maternal interaction, circadian rhythm disorder [37], bronze baby syndrome, overheating with dehydration [38], disturbances in cytokine production [39] or impairment of growth factor receptors [40]. In long-term use, phototherapy may be associated with melanocytic nevi, allergic diseases, patent ductus arteriosus and retinal damage [37].

1.4 Biological impacts of bile pigments

Despite potentially deleterious effects of UCB, bile pigments can also have substantial beneficial effects. In fact, UCB and BV are substances with potential antitumor [41, 42] , anti-inflammatory [43, 44] and anti-oxidant effects [45, 46] with deep inhibitory impact on ROS production. They can even serve as modulators of immune functions [47], cell signaling [48], activators of a AhR [19, 49], inhibitors of protein phosphorylation [50] or NADPH oxidase activity [51]. Even more, current studies are focused on artificial mild elevation of serum UCB concentrations to produce "iatrogenic Gilbert syndrome" with the aim to enhance antioxidant defense. Such approach could be based either on drug intake or by direct administration of UCB, BV or bilirubin-like tetrapyrroles [52, 53]. That may include ingestion of algal biliverdin metabolites, phycobilins, which may undergo reduction by biliverdin reductase [54], and may have antioxidant properties comparable to UCB. Moreover, tetrapyrrole-rich algae, such as *Spirulina platensis* or *Chlorella* belong to popular dietary supplements.

Current research is focused on investigation of diverse biological effects of bile pigments that can finally lead to better understanding and novel treatment strategies of civilization diseases.

1.5 Tetrapyrroles

Tetrapyrroles cover large group of organic compounds that are evolutionary very old and common in Nature. There is a resemblance of plant/cyanobacterial linear tetrapyrroles to bile pigments. BV is believed to be the common precursor in the biosynthetic pathway of

phytochromobilin and different phycobilins [55], such as phycocyanobilin (PCB), phycoerythrobilin, phycourobilin, phycoviolobilin, that are open-chain chromophors representing light harvesting moieties in plants or cyanobacterias. Even more, PCB, phycoerythrobilin and phytochromobilin can be enzymatically converted to rubinoid products by biliverdin reductase [54]. This ubiquitous enzyme catalyzes the reduction of biliverdin to bilirubin in the heme catabolism as described above.

Other extremely important tetrapyrrolic light-harvesting biomolecules are chlorophylls that are present in all green plants, algae and cyanobacterias. Chlorophylls are lipophilic and light sensitive compounds. Due to these reasons, chlorophyllin, a stable and water-soluble artificial derivate of chlorophyll is commonly used in biological experiments (as well as in food industry). Chlorophyll and its derivatives are effective in binding carcinogens such as polycyclic aromatic hydrocarbons, heterocyclic amines, aflatoxin, and other hydrophobic toxins. The chlorophyll-carcinogen complex lowers the bioavailability of carcinogens, which are then more difficult to absorb and can be excreted with the feces [56, 57]. Anti-cancer and chemopreventive effects of chlorophylls have been described in experimental as well as human studies [58-60], but surprisingly, the published data on biological effects of chlorophylls are scarce.

2 Hypothesis and Aims

Objectives of this work were at the beginning focused on the not properly known intestinal metabolism

of bilirubin, especially on the broad enzymatic abilities of nonpathogenic bacterium *C. perfringens*, isolated from human neonatal stool and capable of reducing UCB. Moreover, bilirubin potently affects carcinogenesis of the intestine and its metabolism within the intestinal lumen seems to be crucial for this activity. In our first paper **„Reduction of bilirubin ditaurate by the intestinal bacterium *Clostridium perfringens*“** we examined, whether bilirubin ditaurate, a pigment that naturally occurs in bile of lower vertebrates [61], can be reduced by *C. perfringens* as well, and we aimed to characterize bilirubin ditaurate reduction products.

In the center of our further investigation was to study neurotoxic effects of UCB molecule. Neurotoxicity of bilirubin can manifested in patients with severe unconjugated hyperbilirubinemias, such as Crigler-Najjar syndrome or in neonates with severe neonatal jaundice. Importantly, mechanisms of neurotoxic effects of bilirubin are predominantly identical with those, by which bilirubin inhibits cancer cells growth. The animal model of unconjugated hyperbilirubinemia, jaundiced Gunn rats, was used in our next two studies. In the paper „**Bilirubin accumulation and *Cyp* mRNA expression in selected brain regions of jaundiced Gunn rat pups**“ we aimed to describe the UCB distribution in different brain regions and even more, to yield information about potent protective mechanism of neuronal cells (Cyps and ABC transporters expression) against high UCB levels. The intention of our second experimental study on jaundiced Gunn rats named **“Beyond plasma bilirubin: The effects of phototherapy and albumin on brain bilirubin levels in Gunn rats”** was to assess the

potential role of albumin supplementation in treatment of severe unconjugated hyperbilirubinemias.

The other heme catabolic pathways product, CO, was explored in the next paper **„Antiproliferative effects of carbon monoxide on pancreatic cancer“**. We asked if the pleiotropic impact of the important signal CO molecule can also have antiproliferative impact on aggressive human pancreatic cancer. Furthermore, we were interested in CO distribution within organs and tissue of experimental animals after CO exposure.

Based on recent research work, which often shows connection between higher physiological UCB blood level and lower prevalence of lifestyle diseases, and the structure resemblance of bile pigments to cyanobacterial/plant tetrapyrroles we hypothesized, that the cyanobacterial/plant tetrapyrroles may also have antiproliferative effects. In our last presented paper **„Anti-cancer effects of blue-green alga *Spirulina platensis*, a natural source of bilirubin-like tetrapyrrolic compounds“**, we aimed to examine health protective, antioxidant and antiproliferative impact of the alga *S. platensis* and its tetrapyrroles.

3 Materials and Methods

Cultivation of *C. perfringens*

C. perfringens, isolated from neonatal stool [22] and classified as non-pathogenic, was cultivated under anaerobic condition (37°C,Anaerostat, Oxoid, GB) in 2% yeast extract (Oxoid, GB) buffered with 100 mM phosphate buffer, pH=8.

Isolation of urobilinoids

Urobilinoids were isolated from metabolized medium after 24 hours incubation of *C. perfringens* culture with UCB or BDT (Frontier Scientific) using SPE column (Strata C8 500 mg/6 ml, Phenomenex, CA, USA).

Isolation of UCB from tissues

UCB was isolated from samples of tissue according to Zelenka et al. [62]. UCB was extracted from disintegrated tissue into extraction solvent (methanol/chloroform/n-hexane 63:31:6 (v/v/v)) with mesobilirubin (Frontier Scientific, UT, USA) use as an internal standard.

Isolation of PCB from *S. platensis*

PCB was isolated from lyophilised *S. platensis* according to Terry [63]. The method is based on water extraction and phycobilisome precipitation under acidic condition, non-enzymatic cleavage off PCB with final isolation on SPE column (C18, Sep-Pak, Waters).

Analysis of tetrapyrroles

Gained BDT and UCB reduction products were separated on HPTLC aluminum plates coated with silica gel (RP-18 W/UV254, Macherey-Nagel, Germany) with subsequent analysis of their oxidation products under visible and UV light (CAMAG TLC Scanner II, CAMAG, Muttenz, Switzerland). Urobilin (Frontier Scientific, UT, USA) and urobilinogen ditaurate [64] were used as standards.

Spectrophotometry (UV/Vis Spectrophotometer Lambda 20, Perkin-Elmer, USA) was used for determination of concentration of urobilinoids [65], UCB, BV, and PCB [54].

High performance liquid chromatography (HPLC) analyses of PCB and UCB were performed on Agilent 1200 HPLC instrument with diode array detector (Agilent, Santa Clara, CA, USA) based on a method described previously [62]. The octyl reverse phase column with a safety precolumn (Luna C8, size 4.6x150 mm, particles 3m/100A, Phenomenex, CA, USA) and isocratic mobile phase (methanol/water/tetrabutyl amonium hydroxide, 59:40:1 (w/w/w), pH adjusted to 9.0 by phosphoric acid) were used.

Preparation of water extract from *S. platensis*

Lyophilized *S. platensis* powder was dissolved in distilled water, sonicated, centrifuged and the supernatant was finally lyophilized over-night. Shortly before use, it was dissolved in the culture medium, sterile-filtered and added to cancer cells.

Cell culturing

Human pancreatic adenocarcinoma cell lines - PATU-8902 (DSMZ, Germany), MiaPaCa-2 and BxPC-3 (ATCC, USA) were employed and cultured at 37°C in the atmosphere of 5% CO_2 and 95% air. PATU-8902 and MiaPaCa-2 were grown in Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich) with 10% bovine serum (BS; PAA), 100 units/ml of penicillin (Sigma-Aldrich), 0.01 mg/ml of streptomycin (Sigma-Aldrich). BxPC-3 cell line was cultured in RPMI (Sigma-Aldrich)

with 10% bovine serum (BS; PAA), 100 units/ml of penicillin (Sigma-Aldrich), 0.01 mg/ml of streptomycin (Sigma-Aldrich). All cell lines were authenticated (STR analysis made by Generi Biotech) and regularly tested for Mycoplasma contamination (MycoAlert Detection Kit, Lonza). The cell line PATU-8902 was used in majority of our experiments due to its highest sensitivity to the tested tetrapyrroles. Cells were grown in 96 well plates and incubated with different concentration of UCB (0-100 μmol/L), PCB (0-250 μmol/L) , chlorophyllin (0-500 μmol/L) and extract from *S.platensis* (0-2,5 g/L) for 24 hours, the viability and a cell number was set up, see below.

Viability of cancer cell lines was examined by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide, Sigma-Aldrich) based on [66]. Briefly, the MTT reduction product- formazan, was measured. It is a result of mitochondrial reduction and reflects the metabolic activity- viability of the cell.

Counting of cells cultured in 96 well plate was performed trough crystalline violet staining, that penetrates into the nucleus and stains DNA. Absorbance at 590 nm was measured.

Gene expression analysis

RNA was isolated (PerfectPure RNA Cultured Cell Kit, 5PRIME) from PATU-8902 cells grown in 6 well plates and exposed to UCB (10 μM), PCB (30 μM) and extract from *S. platensis* (0.3 g/L). For cDNA synthesis were used isolated RNA, Random Hexamer Primer, Moloney Murine Leukemia Virus reverse transcriptase (M-MLV, 10 000 units, EastPort), RNase Inhibitor

(RNasin Plus RNase Inhibitor, 10 000 units, EastPort), dNTPs (10 mM), M-MLV Reverse Transcriptase Buffer (5x, EastPort), deionized water.

Real time polymerase chain reaction (RT-PCR) was performed on ViiA 7 instrument (Applied Biosystems) in SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA), and 200-1000 nM of forward and reverse primers. Expressions of vascular endothelial growth factor A, NADPH oxidase 2, p22phox NADPH oxidase, hypoxanthine phosphoribosyl transferase (HPRT) were determined. Data were normalized to HPRT level and expressed in percentage to control.

4 Results and Discussion

In our work **„Reduction of bilirubin ditaurate by the intestinal bacterium *Clostridium perfringens*“** products of the enzymatic process mediated by bilirubin-reducing strain of *C. perfringens* were identified and characterized. It was demonstrated that BDT is reduced without previous amid bond hydrolysis. The conversion rate was substantial, although not as efficient compared to UCB. Based on comparison with bilirubin reduction products and synthetic urobilinogen ditaurate standards, three BDT reduction products were identified: urobilinogen ditaurate, urobilin ditaurate and most likely mesobiliviolin ditaurate. Our results demonstrate very broad enzymatic substrate specificity of bilirubin reducing enzyme(s) and may help in understanding of bilirubin metabolism in the intestinal lumen by intestinal microflora with possible clinical implications including protective effects of bilirubin within the intestinal tract.

Next two studies were focused on UCB neurotoxicity and the animal model of unconjugated hyperbilirubinemia, jaundiced Gunn rats, was used. The paper **„Bilirubin accumulation and *Cyp* mRNA expression in selected brain regions of jaundiced Gunn rat pups“** uniquely describes UCB distribution and mRNA expression of cytochrome P450 monooxygenases and ABC transporters within different brain areas (cortex, superior colliculi, inferior colliculi, cerebellum) of experimental animal model of severe neonatal jaundice. The most sensitive cerebral areas for UCB accumulation were cerebellum and inferior colliculi (parts of the brain responsible for motor coordination and auditory functions). In contrast to cortex and superior colliculi where the UCB accumulation was limited and brief. Interestingly, there was massive and immediate up-regulation of Cyps and immediate ABC transporters expression in UCB unaffected brain regions (cortex, superior colliculi). This was in distinctive contrast to the delayed and relatively small up-regulation of Cyps and ABC transporters in the affected brain regions (cerebellum and inferior colliculi). It seems that the close relationship in distinct brain regions between the extent of UCB accumulation and induction of Cyp mRNA plays an important role in protecting selected brain areas from bilirubin neurotoxicity. It is also believed that the mechanisms causing bilirubin-induced neurotoxicity are likely to be responsible also for beneficial anti-proliferative/anticancer properties.

Data presented in paper entitled **„Beyond plasma bilirubin: The effects of phototherapy and albumin on brain bilirubin levels in Gunn rats“** support adjunct

albumin treatment for severe unconjugated hyperbilirubinemia. Human albumin was administered to chronic (a model for CN syndrome) and acute hemolytic (a model for severe neonatal jaundice) hyperbilirubinemic Gunn rats with phototherapy. Our study confirmed importance of Bf for pathogenesis of bilirubin neurotoxicity, secondly, albumin treatment was demonstrated to enhance the efficacy phototherapy leading to significantly decreased plasma Bf and brain bilirubin. Our results support the feasibility of adjunct albumin treatment in patients with CN syndrome or neonatal jaundice.

Our paper **„Antiproliferative effects of carbon monoxide on pancreatic cancer“** highlights potential anticancer impact of CO, an important gaseous molecule originating from heme catabolism. In our study, CO released from CO releasing molecule or in the form of inhaled gas significantly inhibited proliferation of human pancreatic cancer cells. CO decreased substantially Akt (serin/threonin protein kinase B) phosphorylation in the cancer cells. Athymic mice xenotransplanted with pancreatic tumor and treated with CO had doubled the survival rates with significant inhibition of tumor proliferation and lower density of microvascular net formation. Interestingly, mice exposed to CO led to an almost 3-fold increase in CO content in tumor tissue grown subcutaneously. Additionally, our study reports for the first time the pharmacokinetic data of CO inhaled by experimental animals demonstrating a clinically important CO distribution within the various organs and tissue of the mice body. Results of this work point to the

potential chemotherapeutic/chemoadjuvant use of CO in pancreatic cancer treatment.

Our work presented in the paper **„Anti-cancer effects of blue-green alga *Spirulina platensis*, a natural source of bilirubin-like tetrapyrrolic compounds“** describes antiproliferative effects of edible blue-green algae *S. platensis* and its tetrapyrroles (PCB and chlorophyllin, a surrogate molecule for chlorophyll A) on experimental pancreatic cancer. *In vitro,* a decrease of human pancreatic cancer cell lines´ viability was demonstrated in a dose-dependent manner. The antiproliferative effects of *S. platensis* were also shown *in vivo* in a xenograft mouse model. All tested compounds decreased generation of mitochondrial ROS and modulated glutathione redox status. These data support a chemopreventive role of this edible alga.

5 Conclusion

We pointed at very broad enzymatic equipment of non-pathogenic intestinal bacterium *C. perfringens*, isolated from neonatal stool. It was capable of BDT reducing even without previous deconjugation as well as UCB. Novel BDT reduction products were described. These data might also help to understand the role of intestinal microflora in colonic carcinogenesis.

UCB potential neurotoxicity was studied on the animal model of severe hyperbilirubinemias, such as CN syndrome or pathological neonatal jaundice. UCB brain distribution was monitored and the potent neuronal cells defense mechanisms were suggested. Furthermore human albumin administration during the common hyperbilirubinemias treatment – phototherapy, was

shown to significantly increase the efficiency of phototherapy cure. Our work as well supports the importance of Bf for biological effects of bilirubin. It is believed that the mechanisms causing bilirubin-induced neurotoxicity are likely to be responsible also for beneficial anti-proliferative/anticancer properties.

We investigated other significant heme degradation product, CO, in which we proved the antiproliferative effects on human pancreatic tumors xenotransplanted into the nude mice.

Current research is also focused on the other face of UCB, which is the health-protective impact. Based on these facts, we then investigated the antiproliferative effects of algal, bilirubin-like tetrapyrroles. Indeed, these effects were proved in experimental model of pancreatic cancer.

The presented thesis is focused on not properly described UCB metabolism by intestinal bacteria and highlights important biological impacts of heme catabolic pathway´s products.

6 List of abbreviations

ABC- ATP-binding cassette
ATP- adenosine triphosphate
AhR- aryl hydrocarbon receptor
BDT- bilirubin ditaurate
Bf- free bilirubin
BLVRA- biliverdin reductase
BV- biliverdin
CN- Crigler-Najjar
CO- carbon monoxide
GS- Gilbert syndrome
HPLC- high performance liquid chromatography
HPRT- hypoxanthine phosphoribosyl transferase
MAPK- mitogen-activated phospokinase
MRP- multidrug resistance-related polypeptide
MTT- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide
NAD- nicotinamide adenine dinucleotide
NADP- nicotinamide adenine dinucleotide phosphate
OATPs- Organic Anion Transport Polypeptides
PCB- phycocyanobilin
ROS- reactive oxygen species
RT-PCR - Real time polymerase chain reaction
UCB- unconjugated bilirubin
UGT1A1- UDP-glucuronosyl transferase
UDP- uridin-diphosphate

7 References

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