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Biologický význam metabolických produktů hemu a bilirubinu

Mgr. Jana Jašprová

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Předseda oborové rady:

Prof. MUDr. Stanislav Štípek, DrSc.

Školicí pracoviště: **ÚLBLD, 1. LF UK v Praze**

Školitel: **Prof. MUDr. Libor Vítek, PhD, MBA**

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 Univerzity Karlovy v Praze.

Abstrakt

Předkládaná práce se zabývá studiem významu produktů katabolické dráhy hemu, zejména s ohledem na patogenezi, diagnostiku a léčbu nekonjugovaných hyperbilirubinémií (závažná novorozenecká žloutenka a Criglerův-Najjarův syndrom). Jedním z hlavních cílů bylo ozřejmění biologických účinků produktů bilirubinu, které vznikají při fototerapii těchto onemocnění a otestování nových léčebných přístupů a to jak na úrovni genové terapie, tak farmakoterapie.

Novorozenecká žloutenka je jednou z nejběžnějších komplikací v neonatálním období. Zlatým standardem v její léčbě je fototerapie modrým světlem, jejíž použití však může být doprovázeno i závažnými nežádoucími efekty. Nutno podotknout, že fototerapie novorozenecké žloutenky je v některých zemích nadužívána, a že pacienti s Criglerovým-Najjarovým syndromem typu I jsou vystaveni celoživotní fototerapii (pokud nepodstoupí transplantaci jater).

V rámci předkládané práce jsme na experimentálním *in vitro* modelu studovali biologické účinky fotoizomerů bilirubinu, které vznikají v průběhu terapie novorozenecké žloutenky. Dále jsme se za použití experimentálních modelů hyperbilirubinemických potkanů a myší zabývali možnostmi zavedení vhodné genové terapie, kterou by bylo možné bezpečně použít v léčbě Criglerova-Najjarova syndromu a omezit nebo zcela odstranit nutnost celoživotní fototerapie, a dalších terapeutických modalit, jako jsou výměnná transfúze a aplikace lidského sérového albuminu v léčbě Criglerova-Najjarova syndromu a novorozenecké žloutenky.

Abstract

Present work has been focused on the importance of the products of the heme catabolic pathway, in particular under conditions of unconjugated hyperbilirubinemias (neonatal jaundice and Crigler-Najjar syndrome (CNS)). The second part of the project was focused on the improvement of some pharmacological approaches used in the treatment of these diseases, as well as on studies of bilirubin products that are formed during the treatment by phototherapy (PT).

Neonatal jaundice is one of the most common complications in neonates. Currently, there is no efficient pharmacotherapy and the treatment with blue light is used as a gold standard for severe neonatal jaundice. However, the absolute safety of PT has still not been confirmed. In this context, it is important to note that some neonatologists start the PT before serum bilirubin levels reach the recommended values and that patients with CNS type I (CNSI) are forced to be on life-long PT (unless undergoing liver transplantation).

The focus of the present project was to study biological effects of bilirubin photoisomers (PI) in an *in vitro* model of the human neuroblastoma SH-SY5Y cells that are used for studies of the neuronal metabolism. In further studies performed on animal model of hyperbilirubinemic rats and mice, we investigated a suitable gene therapy to be used in CNSI patients with the aim to reduce or eliminate the need of PT. Finally, we have compared the efficacy of PT, exchange transfusion (ET) and human serum albumin administration (HSA) in the therapy of CNSI and severe neonatal jaundice with respect to determination of free bilirubin (Bf) levels and bilirubin concentrations in various brain tissue compartments in the hyperbilirubinemic Gunn rats.

Content

1 Introduction

1.1 Haem catabolism

Haem plays a key role in multiple functions in the human body and is clearly essential for life (1). Haem, or iron protoporphyrin, is a cyclic tetrapyrrole with the centrally bound atom of iron (2), being ubiquitously expressed in the majority of tissues (3).

Once haem is released from the red blood cells it is bound to haemopexin or haptoglobin and recycled (4) or transported to the reticuloendothelial system where it is degraded through the haem catabolic pathway into linear yellow tetrapyrrole, unconjugated bilirubin (UCB), by the action of two enzymes, haem oxygenase (HMOX) and biliverdin reductase (BLVR) (for review see (2)). This process takes place in all tissues, predominantly in spleen, and serves for elimination of otherwise toxic free haem with pro-oxidant effects (5). Main part of haem degradation takes place in reticuloendothelial system of spleen, bone marrow and liver (6).

1.2 Biological effects of bilirubin

Bilirubin is one of the most powerful endogenous free radical scavengers not only for reactive oxygen species (ROS), but also reactive nitrogen species (RNS) and other NO-related reactive compounds (7).

Other beneficial bilirubin actions, such as anti-oxidant, anti-inflammatory and cytoprotective effects, have been then confirmed in numerous studies (8-10). It has also been proven that mildly elevated bilirubin concentrations in the body could help to lower the risk of cancer, cardiovascular diseases and other oxidative stress-mediated diseases (11).

Less than 0.1 % of the concentration of UCB in plasma is not bound to any carrier molecule and is called free bilirubin (Bf) (12). Thanks to its lipophilic nature, this albumin-unbound fraction has a high permeability through the lipid bilayer membranes, and thus is able to cross the blood-brain barrier (13). It is believed that Bf is responsible for toxic effects of bilirubin (14) and is even a better predictor for evaluation of bilirubin neurotoxicity (14-16).

1.3 Hyperbilirubinemias

Physiological concentrations of bilirubin in plasma/serum are normally around 10 μmol·L^{-1} with the reference range up to 17 μmol·L^{-1}. Once bilirubin levels in the circulation rise above physiological concentrations, icteric discoloration of sclera, mucosal surfaces and skin is observed.

Mildly elevated systemic bilirubin levels, such as in subjects with Gilbert syndrome, are associated with protection from development of various oxidative stress-mediated diseases, such as atherosclerosis and cancer (5). Much more severe hyperbilirubinemias (usually above 340 μmol·L^{-1} in newborns, and even higher in adults) could be accompanied with deleterious bilirubin effects, among them kernicterus and bilirubin-induced neurological dysfunction being the worst complications (17).

Hyperbilirubinemias are classified according to type of bilirubin that is elevated into unconjugated (pre-microsomal), conjugated (post-microsomal) and mixed hyperbilirubinemias (18). A wide range of genetic factors that may predict the incidence of hyperbilirubinemias.

1.3.1 Neonatal jaundice

Neonatal jaundice is defined as a condition accompanied with total serum bilirubin levels above 85 μmol·L^{-1} (corresponding to 5 mg·dL^{-1}) (19). Almost 60 % of term and 80 % of preterm newborns are visibly jaundiced in their first days of life (20-22).

The pathogenesis of neonatal jaundice is multifactorial, and is due to imbalance between production and elimination of bilirubin after birth (23). Among many others, factors, such as ABO and Rhesus factor blood group incompatibility, deficiency of glucose-6-phosphate dehydrogenase, sepsis, newborn immaturity or even breast feeding are the most important ones (24). Neonatal hyperbilirubinemia may lead to bilirubin accumulation in basal ganglia and brain stem nuclei and thus lead to chronical or acute bilirubin neurotoxicity (25).

Many therapeutic approaches have been proposed and attempted in the past for the treatment of neonatal jaundice, but PT is still believed to be the gold standard in the therapy.

1.3.2 Crigler-Najjar syndrome

CNS is another type of unconjugated hyperbilirubinemia, which is extremely rare (26). It is classified, based on bilirubin levels as CNSI and CNSII (27, 28). The liver histology of patients suffering from CNS is without any pathology, and the patients have normal hepatic metabolic function except for bilirubin glucuronosylation (29).

CNSI is caused by the lack of hepatic bilirubin UDP-glucuronosyl transferase (UGT1A1) activity. Because of that, bile contains only traces of bilirubin conjugates, serum bilirubin levels are higher than 340 $\mu mol \cdot L^{-1}$. On the other hand, patients with CNSII have some residual activity of UGT1A1. Both types of the disease are extremely rare; the prevalence of both types reaches only 1 case *per* 1,000,000 of newborn infants (30), but could differ geographically.

The main treatment option is PT needed throughout the whole life; the only curative option is the liver transplantation (31) which is used mainly in patients with CNSI. Due to low availability of the liver grafts, as well as invasiveness of this therapeutic approach, there is still need for searching of alternative treatment option. These include, for instance gene therapy (31), or approaches focused on interruption of enterohepatic cycling of bilirubin (32).

A natural animal model for the CNS is represented by the hyperbilirubinemic Gunn rats identified by Gunn in 1934 (33).

1.4 Treatment options in unconjugated hyperbilirubinemias

Unconjugated hyperbilirubinemia is a treatable phenomenon. Current clinical practice for treatment neonatal jaundice is PT and/or ET (34). In the treatment of CNS, long-term PT (12 and more hours per day) and liver transplantation is being used (31). A wide range of other treatment options have been proposed and tested under experimental as well as clinical conditions, but most of them did not proceed into clinical use.

1.5 Bilirubin-derived products

1.5.1 Bilirubin photoisomers

By the action of blue or blue-green light used during PT of neonatal jaundice, bilirubin is transformed into its structural and geometrical photoproducts which are called bilirubin PI (35). Configurational isomerisation of bilirubin leads to the formation of ZE- and EZ-bilirubin, this change is reversible and much faster than the structural isomerisation that leads to irreversible change of bilirubin into E- and Z-lumirubin (36). These bilirubin products are more polar and could be easier excreted from the body (37).

The photoreactivity of bilirubin has been studied since 1970’s, the first review on this topic wrote Lightner in 1977 (38). Exact structures of bilirubin PI were established by McDonagh et al. (39) and Onishi et al. (40). Same authors have concluded that Z-lumirubin is the most important bilirubin PI (40). Although it is generally believed, that

bilirubin PI are non-toxic, the data on their potential biological activity and proper mechanisms are still lacking.

Bilirubin PI could be detected by high-performance liquid chromatography (HPLC) (41, 42) in bile, serum and urine, but none of these methods is being used in clinical practice.

1.5.2 Bilirubin oxidation products

Bilirubin may act as an antioxidant by scavenging reactive oxygen species; in this process and during PT of neonatal jaundice bilirubin oxidative metabolites are formed (43). These metabolites are divided into tripyrrolic (biopyrrins), dipyrrolic (propent-dyo-pents) and monopyrrolic (bilirubin oxidation products) degradation products.

2 Hypothesis and Aims

First part of this work was focused on clarification of effects of compounds formed from bilirubin during PT, which are known as bilirubin PI. Second part of our investigations was oriented on searching new therapeutic approaches for severe unconjugated hyperbilirubinemias typical for CNS and neonatal jaundice. Therapeutic approaches were divided into gene therapies and the role of HSA in the treatment of neonatal jaundice.

Because of the lack of commercially available standards of bilirubin PI, ZE-/EZ-bilirubin and lumirubin, there is still no consensus with respect to their potential toxicity. Goal of our paper called **“The biological effects of bilirubin photoisomers”** was to isolate bilirubin PI in pure forms and to test their potential biological effects *in vitro* on human neuroblastoma SH-SY5Y cells.

Main goal of the paper named **“Photo-isomerization and oxidation of bilirubin in mammals is dependent on albumin binding”** was to study binding sites for bilirubin and its derivatives in the structure of HSA by using methods of circular dichroism, fluorescence spectroscopy and molecular modelling.

In case of CNSI, patients need long-term PT or liver transplantation. As an alternative approach, a gene therapy is considered a promising treatment tool. The aim of our study entitled **“Sustained reduction of hyperbilirubinemia in Gunn rats after adeno-associated virus-mediated gene transfer of bilirubin UDP-glucuronosyltransferase isozyme 1A1 to skeletal muscle”** was to investigate the preclinical safety and efficacy of muscle-directed deficient gene transfer mediated by adeno-associated viral (AAV) vectors for the therapy of CNSI.

Study named **“Life-long correction of hyperbilirubinemia with a neonatal liver-specific AAV-mediated gene transfer in a lethal mouse model of Crigler-Najjar syndrome”** was focused on assessment of the therapeutic effect of the AAV vector injection as well as comparison of the efficacy of liver versus skeletal muscle specific transgene expression. For this investigation Ugt1 mutant mice were used.

Helper-dependent adenoviral (HDAd) vectors should be more suitable for gene therapy than adenoviral vectors (44). In our paper **“Improved efficacy and reduced toxicity by ultrasound-guided intrahepatic injections of helper-dependent adenoviral vector in Gunn rats”** we tested their effects on the expression of UGT1A1. We studied the effect of the vector dosage on the expression of UGT1A1 and searched for that one that would be free of toxicity, but still able to reduce bilirubin levels.

Another approach to treatment of unconjugated hyperbilirubinemias lies in pharmacotherapy. The most logical option is the administration of HSA to increase its pool in the circulation and thus reduce Bf responsible for neurological damages (12). The aim of a study entitled **“Beyond plasma bilirubin: The effects of phototherapy and albumin on brain bilirubin levels in Gunn rats”** was to evaluate the effect of HSA treatment, PT and combination of these two therapeutic approaches in acute and chronic models of unconjugated hyperbilirubinemia.

In the study **“Albumin administration protects against bilirubin-induced auditory brainstem dysfunction in Gunn rat pups”** we focused on the potential therapeutic role of HSA administration in a rat model of acute hyperbilirubinemia inducted by haemolysis or bilirubin-albumin displacement.

In addition to PT, ET is the other treatment option for neonatal jaundice. Our goal in **“Optimizing exchange transfusion for severe unconjugated hyperbilirubinemia: Studies in the Gunn rats”** was to optimize the conditions of ET in Gunn rats and compare its efficacy with PT, HSA administration and the combination of each of them with ET.

Because we found out that HSA administration is able to decrease bilirubin levels in circulation as well as in selected organs, we performed a study entitled **“Albumin administration prevents neurological damage and death in a mouse model of severe neonatal hyperbilirubinemia”,** in which we treated mutant *Ugt1* mice with repeated HSA doses without PT. We wanted to show whether daily administration of HSA could prevent bilirubin induced neurotoxicity in our murine model.

3 Materials and Methods

Purification of bilirubin

Commercial bilirubin (Applichem, Germany) was purified prior the usage according the modified McDonagh's method (45).

Isolation of tissue bilirubin

Bilirubin was isolated from tissues by the method according to Zelenka *et al.* (46). Homogenized tissue was extracted by methanol/chloroform/hexan (40/20/4 v/v/v) with mesobilirubin as an internal standard. Extracted sample was concentrated into small droplet of carbonate buffer (pH 10) and subsequently analysed by HPLC.

Isolation of bilirubin PI

Mixture of bilirubin PI from samples of serum were isolated according to McDonagh (47) with 0.1 $mol \cdot L^{-1}$ di-n-octylamine acetate in methanol and analysed by HPLC.

Bilirubin PI *per se* were isolated by modification of methods of Stoll *et al.* (48, 49) and Bonnett *et al.* (50). Prepared mixture of all bilirubin photoderivatives was separated and isolated by thin layer chromatography (TLC).

Isolation of mono- and bisglucuronosyl conjugates

Bilirubin conjugates were isolated from bile according to Spivak and Carrey (51) and their amounts were determined by HPLC.

HPLC

All HPLC analysis were performed on HPLC system Agilent 1200 (CA, USA) equipped with a diode-array detector.

Tissue bilirubin was determined by an HPLC method according to Zelenka *et al.* (46) using an analytical column Luna C8 (4.6 mm x 150 mm, particles 3 μm/100 A; Phenomenex, CA, USA) and a mixture of water/methanol/tetrabutylammonium hydroxide as a mobile phase. The signal was stored at 440 nm with 550 nm as the reference wavelength.

Analysis of bilirubin PI was performed by a modified method according to McDonagh *et al.* (47) on the Poroshell SB-C18 column (4.6 mm x 100 mm, 2.7 μm particles; Agilent, CA, USA) with the mobile phase composed of 0.1 $mol \cdot L^{-1}$ di-n-octylamine acetate in methanol and water in a different ratio (92:8 or 90:10, v/v). The signal was stored at 453 nm.

Analysis of bilirubin PI and conjugates was performed by a modified method according Spivak and Carey (51) on the Purospher RP-18 column (4 mm x 250 mm, 5 μm particles; Merck, Germany) with gradient elution of methanol into 1 % ammonium acetate (pH 4.5). The signal was stored at 450 nm.

Mass spectroscopy (MS)

MS analyses of bilirubin PI were performed on Escuire 3000 mass spectrometer (Bruker Daltonics, Germany) coupled with electrospray ionisation. The measurement was provided in a negative mode. The masses were scanned in the range between 50 and 800 m/z. The capillary exit was set at -106.7 V.

TLC

TLC was used for preparation of pure bilirubin PI. Photo-irradiated mixture of bilirubin was dissolved in methanol/chloroform (1:1, v/v) and injected onto silicagel plate (Kieselgel 60; Merck, Germany). The plate was developed in a mobile phase composed of chloroform/methanol/water (40:9:1, v/v/v).

Determination of Bf

Effect of bilirubin PI on Bf levels was studied by a peroxidase method (14). For the determination of Bf in brains, correction of tissue bilirubin and tissue albumin was used. Protein from the brain was isolated according to Ericsson *et al.* (52) and the albumin content was determined by an ELISA kit for rat albumin (E91028Ra, USCN, TX, USA).

Cell cultures

The effect of bilirubin PI was tested on human neuroblastoma cell line SH-SY5Y (ATCC, USA) used as a standard model for studies on metabolism of neuronal cells.

Cells were exposed to a medium containing bilirubin (24 $\mu mol \cdot L^{-1}$), bilirubin and bilirubin PI (24 $\mu mol \cdot L^{-1}$ UCB + 5, 15 or 30 % PIs), pure bilirubin PIs (5, 15 or 30 %) and DMSO prior performing viability tests, mRNA and FACS analysis.

Cell viability was determined by different methods. MTT and XTT were used as the standard screening methods. In addition, more accurate fluorescent CellTiter-Blue Assay (Promega, USA) and luminescent CellTiter-Glo (Promega, USA) were used, as well.

Gene Expression Analysis

Cells were seeded onto 6-well and treated as described above for 4 and 24 hours. RNA was isolated by using PerfectPure RNA Cell kit (5Prime, USA) and transcribed by High-Capacity cDNA reverse transcription kit (Life Technologies, USA).

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

4 Results and Discussion

This Thesis was focused on the evaluation of the effects of bilirubin and its isomers that are produced during PT of neonatal jaundice as well as on different therapies for the treatment of unconjugated hyperbilirubinemia.

In our paper entitled **"The biological effects of bilirubin photoisomers"** we isolated bilirubin PI – ZE-/EZ-bilirubin and lumirubin – in their pure forms, and tested their biological effects *in vitro* on human neuroblastoma SH-SY5Y cell line. We found out that albumin had a binding site for lumirubin that was different from that for bilirubin and it was located in the subdomain IB. Lumirubin had much lower binding constant than bilirubin for albumin and it did not affect concentration of Bf. In comparison to bilirubin, bilirubin PI did not influence the viability of studied cell line and were not able to change the expression of genes involved in the its cell cycle regulation or the haem catabolic pathway.

In the study **"Photo-isomerization and oxidation of bilirubin in mammals is dependent on albumin binding"** the binding sites for bilirubin, its derivatives (mesobilirubin, bilirubin ditaurate), PI and oxidation products (lumirubin, biliverdin and xanthobilirubic acid) were characterized on HSA using a combination of circular dichroism, fluorescence spectroscopy and molecular modelling methods. We discovered that bilirubin and its products bound to two independent binding sites.

Goal of our study entitled **"Sustained reduction of hyperbilirubinemia in Gunn rats after adeno-associated virus-mediated gene transfer of bilirubin UDP-glucuronosyltransferase isozyme 1A1 to skeletal muscle"** was to evaluate the role of gene therapy as a potential treatment option for CNS. Rat *Ugt1a1* expressing AAV vector was injected directly into muscles of Gunn rats. By this method, the concentration of serum

bilirubin sustained lowered for at least one year period. We also analysed urine and bile and we found there higher elimination of bilirubin metabolic products.

In the paper etitled **"Life-long correction of hyperbilirubinemia with a neonatal liver-specific AAV-mediated gene transfer in a lethal mouse model of Crigler-Najjar syndrome"** we studied the effect of gene transfer of the liver specific AAV vector encoding the *hUGT1A1* gene in the lethal mouse model for CNS. Upon the successful application we were able to see the therapeutic effect for the extended period of 17 months. We also compared the efficacy of the vector's application into liver *versus* skeletal muscle. In case of liver-targeted application, expression of *Ugt1a1* increased for 5-8% of the expression of normal healthy liver and we observed significant decrease of bilirubin. On the other hand, application of the vector that should target the skeletal muscle was not able to decrease bilirubin levels even though the *Ugt1a1* expression rose to 20-30% of normal liver expression. The reason of the observed difference from the previous study could lie in the application approach (intravenous injection of muscle-specific vector *vs.* direct intramuscular application).

Different type of gene therapy was used in our work entitled **"Improved efficacy and reduced toxicity by ultrasound-guided intrahepatic injections of helper-dependent adenoviral vector in Gunn rats".** We used helper-dependent adenoviral (HDAd) vector that did not contain viral coding sequences in comparison to above used adenoviruses, and thus could provide stable and long-term expression of the *UGT1A1* gene. We compared the efficacy of different application modalities on the reduction of hyperbilirubinemia, which was the highest when using the ultrasound-guided intrahepatic application of HDAd vector in the concentration 10^{11} vp/kg.

In our paper entitled **"Beyond plasma bilirubin: The effects of phototherapy and albumin on brain bilirubin levels in Gunn rats"** we tried to clarify whether HSA application may improve in the treatment of neonatal jaundice or CNS. We compared the effect of a single HSA application on the serum bilirubin, Bf and tissue bilirubin concentrations with the usage of PT and combination of both approaches. We observed that single HSA application is able to significantly increase the efficacy of PT.

In the follow-up study entitled **"Albumin administration protects against bilirubin-induced auditory brainstem dysfunction in Gunn rat pups"** we assessed the effect of HSA application on the parameters of the auditory system being impaired in severe neonatal jaundice. We used electroencephalography to detect brainstem auditory evoked potentials (BAEPs). Using an animal model of unconjugated hyperbilirubinemia, we demonstrated that

the HSA treatment is neuroprotective and protects against bilirubin-induced BAEPs. This treatment also tended to reduce Bf in brain.

In our study entitled **"Optimizing exchange transfusion for severe unconjugated hyperbilirubinemia: Studies in the Gunn rats"** we focused on the evaluation of different modalities for treatment of neonatal jaundice, such as PT, HSA and their combinations. After successful establishment of the ET model in Gunn rats, we found out that this method is highly effective in decreasing bilirubin as well as Bf concentrations. Both PT and HSA treatments were found to potentiate the effect of ET. Our optimized *in vivo* animal model for ET should be helpful in further studies investigating treatments of acute hyperbilirubinemias.

In our paper entitled **"Albumin administration prevents neurological damage and death in a mouse model of severe neonatal hyperbilirubinemia"** genetically modified mice lacking the *Ugt1* gene were used to assess therapeutic potential of HSA. Mice were treated with intraperitoneal application of HSA of different doses every 24 or 48 hours immediately after birth. Lower Bf levels in comparison to controls were detected in the circulation of mice treated by HSA. The treated group had higher total serum bilirubin, but on the other hand, significantly lower tissue bilirubin concentrations were found in comparison to controls. Our data indicate that the effect of HSA treatment is highly dependent on its dosage and frequency. Hence, this type of treatment seems to be useful in patients with extreme neonatal jaundice or CNS.

5 Conclusion

We have successfully isolated bilirubin PI in pure forms and clarified some of their biological effects *in vitro*. Our data suggests that bilirubin PI are not responsible for rare complications that could accompany PT of neonatal jaundice. We have mapped the binding sites for bilirubin and its derivatives in the molecule of HSA.

In the second part of our studies we focused on the development of new gene therapeutic approaches for treatment of severe CNSI. We compared the efficacy of different doses of AAV or HDAd vectors and we compared muscle and liver specific and directed treatments. In all our models we were able to successfully increase the activity of UGT1A1 enzyme and to lower systemic hyperbilirubinemia resulting in protection of brain from severe damage. The liver-directed gene therapy seems to be a promising therapeutic approach for CNS; nevertheless, clinical trials are needed to confirm our preclinical data.

In the last part of this work we established an *in vivo* model for ET and compared the efficacy of ET, PT and HSA administration. ET is the most effective way how to treat

neonatal jaundice. However, due to high risk of mortality we propose HSA administration to be a safer way of treatment. Based on our results it is evident that HSA can serve as a supportive therapeutic option to increase the efficacy of PT.

6 List of abbreviations

AAV ... adeno-associated viral vector

BAEPs ... brainstem auditory evoked potentials

Bf ... free bilirubin

BLVR ... biliverdin reductase

cDNA ... complementary deoxyribonucleic acid

CNS (CNSI, CNSII) ... Crigler-Najjar syndrome (type I, II)

ELISA ... enzyme-linked immunosorbent assay

ET ... exchange transfusion

HDAd ... helper-dependent adenoviral vector

HMOX ... haem oxygenase

HPLC ... high-performance liquid chromatography

HSA ... human serum albumin

MS ... mass spectroscopy

MTT ... 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide

PI ... photoisomers

PT ... phototherapy

RNA ... ribonucleic acid

RNS ... reactive nitrogen species

ROS ... reactive oxygen species

RT-PCR ... real time polymerase chain reaction

TLC ... thin layer chromatography

UCB ... unconjugated bilirubin

UGT1A1 ... uridindiphosphate glucuronosyl transferase

XTT ... 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

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8 Published papers

Papers related to the thesis with impact factor

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