



**UDP-GLUCURONOSYLTRANSFERASE (UGT) 1A1 EXPRESSION IN SKIN
AND ROLE OF UVB IN INDUCTION OF UGT1A1 IN THE NEONATAL
HYPERBILIRUBINEMIA**

*Bijay Kumar Sah and Prof. Dr. Zhao-Lin

Dept. of Pediatrics, The 2nd Affiliated Hospital of Kunming Medical University, Kunming Yunnan 650032, China

ABSTRACT

This study is to determine the expression pattern of human UGT1A family enzymes in human skin. This study revealed a protective role of UGT1A1 expressed in the skin against neonatal hyperbilirubinemia. Sunlight, a natural and free source of light, makes it possible to treat neonatal jaundice without phototherapy units while allowing mothers to breast-feed neonates.

Key words: UGT, UGT1A1, Sunlight, HaCaT cells, Neonatal Hyperbilirubinemia,

INTRODUCTION

UDP Glucuronosyltransferase (UGTs) are a family of membrane-bound enzymes that catalyze the transfer of the glucuronic acid moiety of UDP-glucuronic acid to a large number of endogenous and exogenous compounds. Human UGTs are divided into two distinct families, UGT1 and UGT2, on the basis of evolutionary divergence and homology [1]. The UGT1 gene is located on chromosome 2q37 and produces nine functional enzymes (UGT1A1, UGT1A3–UGT1A10) by exon sharing. The UGT2A and UGT2B genes are located on chromosome 4q13, encoding three and seven functional proteins, respectively. The UGT2A1 and UGT2A2 are formed by differential splicing of variable first exons and common exons 2–6, likely the UGT1A gene [1]. Meanwhile, UGT2A3 and each UGT2B are encoded by individual genes [1]. Each UGT enzyme expresses in a tissue-specific manner and exhibits substrate specificity [2].

Bilirubin is an end product of heme catabolism, formed by the breakdown of red blood cells. Bilirubin is taken up from blood into hepatocytes by sinusoidal membrane transporters and then excreted into bile through the bile canalicular membrane where it is conjugated by UGT1A1 with glucuronic acid. (A) Mechanism of bilirubin uptake into hepatocytes: many organic anions are incorporated into hepatocytes by organic anion transporting polypeptides (rat, oatp1, oatp2, oatp3; human, OATP), liver-specific transporter (rlst/HLST), and/or by organic anion transporters (OAT2, OAT3). Oatp1 and HLST transport bilirubin monoglucuronide. However, a transporter of unconjugated bilirubin in the sinusoidal membrane has not as yet been identified. Unconjugated bilirubin may also go across the hepatocyte sinusoidal membrane by a diffusion process. (B) The conjugated bilirubin is excreted into the small intestine via the bile duct [3]. Because UGT1A1 is the sole bilirubin conjugating enzyme [4]. Its genetic polymorphism or inhibition of UGT1A1 activity can cause increased serum levels of unconjugated bilirubin [5-6]. The unconjugated bilirubin crosses the blood-brain barrier and causes kernicterus, which is severe neurologic damage resulting from bilirubin toxicity [7]. Developmentally induced bilirubin toxicity leads to a paradigm of toxic responses in newborns that becomes clinically apparent as lethargy, ophthalmoplegia (ocular muscle paralysis), high-pitch crying, opisthotonus (bowed body and rigid extremities or dystonia), and seizures as well as mental retardation, long-term physical impairment, and often death [8-9]. In addition, severe hyperbilirubinemia can be brought on by infection, ischemia, biliary obstruction, and breastfeeding with inadequate intake as well as a metabolic deficiency in glucose-6-phosphate dehydrogenase (G6PD) activity [10-11]. Among the most severe of the genetic deficiencies, Crigler–Najjar type 1 (CN1) disease is characterized by a complete inactivation of UGT1A1-dependent bilirubin glucuronidation activity [12]. The complete loss of UGT1A1 activity in CN1 is often fatal, resulting from the progression of hyperbilirubinemia to CNS toxicity [13]. Gilbert's syndrome is the most common inheritable condition resulting in transient unconjugated hyperbilirubinemia [14-15]. To prevent occurrences of kernicterus, infants who develop severe hyperbilirubinemia are often treated with phototherapy, through bilirubin isomerization that changes trans-bilirubin into the water-soluble cis-

bilirubin isomer [16-17]. However, the limitation with phototherapy is that it requires mothers not to breast-feed, additionally, it requires conventional phototherapy units, which are not available in some countries.

For the alternative choice for the treatment of neonatal hyperbilirubinemia, sunlight has also been suggested which is shown to be more effective in bilirubin isomerization. [18] Furthermore, aryl hydrocarbon receptor (AhR) which is a nuclear receptor seen in the expression of UGT1A1, is activated by the 1-tryptophan which results from the photo oxidation of UVB in sunlight. [19-20]. Since the skin covers a surface area of approximately 1.7 m² in an average adult body and 0.2 m² in a 3-kg newborn infant, and it receives about one-third of the circulating blood, UGT1A1 expressed in the skin play an important role in sunlight-induced reduction of serum bilirubin.

The expression pattern of UGT1A1 in human skin has not been studied in details. To investigate the protective role of UGT1A1 in the skin against neonatal hyperbilirubinemia, we examined mRNA expression patterns of human UGT1 family enzymes in human skin, human skin keratinocyte (HaCaT) cells, and in recently developed humanized UGT1(hUGT1) mice [21]. We also examined the effects of UVB irradiation on the expression of human UGT1 in HaCaT cells.

MATERIALS AND METHODS

Identification of UGT Isoforms Expressed in Human Skin:

The skin is one of the body's largest primary barriers to the environment, but the expression pattern of detoxification enzymes such as the UGTs in human skin has not yet been quantitated. The expression levels of human UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10 in HaCaT cells [22]. The reverse-transcription polymerase chain reaction (RT-PCR) revealed that a wide variety of UGT1A isoforms, UGT1A1, UGT1A3, UGT1A4, and UGT1A8, were expressed in the HaCaT cells. Expression of human UGT1A mRNA in human also shows expression of UGT1A1, UGT1A3, UGT1A4, and UGT1A8, we observed that UGT1A7 and UGT1A10 were highly expressed in human skin.

The expression pattern of human UGT1A family enzymes in human skin. A 1- μ l portion of complementary DNA reverse-transcribed from HaCaT total RNA (A) or human skin RNA (B)

Induction of UGT1A1, UGT1A8, and CYP1A1 by Irradiated l-Tryptophan in HaCaT Cells:

It has been reported that UVB irradiation photo-oxidizes tryptophan in the skin and produces 6-Formylindolo 3, 2-b Carbazole (FICZ), which is an agonist of aryl hydrocarbon receptor (AhR), which is a nuclear receptor seen in the expression of UGT1A1 [23]. To understand whether UVB irradiation of l-

tryptophan could result in an activation of AhR in the HaCaT cells, inducibility of UVB-irradiated l-tryptophan on CYP1A1, UGT1A1 and UGT1A8 in human skin HaCaT cells was studied. The level of CYP1A1 induction was even higher when HaCaT cells were treated with l-tryptophan that was irradiated for 5 minutes) Since UGT1A1 and UGT1A8 play important roles in the detoxification of bilirubin and carcinogens, similar pattern was seen in the induction pattern of UGT1A1 in HaCaT cells with UVB-irradiated l-tryptophan to that of CYP1A1.

Induction of CYP1A1, UGT1A1, and UGT1A8 in the FICZ-Treated HaCaT Cells and Induction of Estradiol 3-O-Glucuronidation Activities by UVB and FICZ in HaCaT Cells:

HaCaT cells were treated with 6-Formylindolo 3, 2-b Carbazole (FICZ) and RT-PCR was carried out for these genes and studies which show a dose-dependent induction of CYP1A1. The semi quantitative RT-PCR revealed that UGT1A1 and UGT1A8 were induced by significant number in the HaCaT cells. Other study conducted a quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis of UGT1A1 and UGT1A8 in control and FICZ-treated HaCaT cells also show better induction in FICZ controlled study. Similar to CYP1A1, UGT1A1 was also induced by FICZ dose dependently. The study reveals that not only CYP1A1 but also multiple UGT1A isoforms, especially UGT1A1 and UGT1A8, were induced by FICZ in the human skin cells.

Estradiol 3-O-glucuronidation is one of the metabolic reactions selectively metabolized by UGT1A1. Study was done to investigate whether the treatment of the HaCaT cells with UVB-exposed tryptophan and FICZ results in an increase in UGT activities which shows positive result with increase microsomal activity. The fold induction of the UGT activity was correlated with the induction level of UGT1A1 mRNA indicating that UVB or FICZ similarly induced both UGT1A1 mRNA and activity in the HaCaT cells^[24].

Comparison of UGT1A1 Expression in the Skin and Liver:

The importance of UGT1A1 expressed in the skin in neonatal hyperbilirubinemia, we determined and compared the UGT1A1 expression levels in the skin and liver of neonatal hUGT1mice. Whereas in adults, UGT1A1 was mainly expressed in the liver. In neonates, the expression of UGT1A1 was greater in the skin. With increasing days after birth, the expression of UGT1A1 in the skin shows significant increment than that in the liver^[25]. This indicates that in neonatal life, UGT1A1 expressed in the skin might be important in reducing serum bilirubin to prevent the onset of kernicterus.

Effects of UVB Irradiation on UGT1A1 Expression and Activity in Neonatal hUGT1 Mice:

Investigation was done showing whether UVB irradiation results in an induction of skin UGT1A1 which reduces the bilirubin level, as well as study of UGT1A1 mRNA, protein, and activity in the skin. After

certain period, treatment with the UVB shows decrease in the total serum bilirubin level suggesting that the UVB might have induced UGT1A1 expression in the skin to accelerate bilirubin metabolism. QRT-PCR analysis revealed that the UGT1A1 level in the skin of UVB-treated mice was 3-fold higher than the level in the skin of nontreated mice. To examine the effect of the UVB treatment on UGT1A1 protein expression in the skin, skin microsomes were prepared from the control and UVB-treated mice and subjected to immunoblot analysis, which shows very faint expression of the UGT1A1 protein in the skin from hUGT1 mice. With the UVB treatment of the mice, UGT1A protein expressions increased compared with the level in control mice. UGT1A1 activities in the skin microsomes were further assessed to examine whether the induced UGT1A1 mRNA/protein expression resulted in an increase in functional UGT1A1. The estradiol 3-*O*-glucuronide formation rate shows increment in the UVB-treated hUGT1 mice than control, indicating that UGT1A1 activities in the skin were induced with UVB. Study also suggests that the UVB treatment of the mice induced UGT1A1 only in the skin. This finding indicates that the reduction in serum bilirubin levels in the UVB-treated mice can be attributed mainly to the increased expression of UGT1A1 in the skin [24].

DISCUSSION

The expression pattern of phase I and phase II drug-metabolizing enzymes in the liver has been widely analyzed [12, 26]. Meanwhile, extrahepatic tissues such as the skin, the small and large intestine, the kidneys, and the lungs also contribute to the metabolic process of a wide variety of chemicals. Because the skin covers a surface of approximately 1.7 m² in an average adult and receives about one-third of the blood supply, it plays an important role in the metabolic clearance and metabolic activation of endogenous and exogenous compounds. The study indicates the presence of functional UGT enzymes in the skin. In the present study, we performed RT-PCR and real-time RT-PCR from HaCaT cells and human skin, finding that various UGT1A family enzymes, including UGT1A1, UGT1A3, UGT1A4, UGT1A7, UGT1A8, and UGT1A10, were expressed. This finding indicates that although the HaCaT cell line is a spontaneously immortalized human keratinocyte cell line, the UGT1 gene expression pattern in HaCaT cells is very similar to that in actual human skin.

Neonatal jaundice is a condition marked by high levels of serum bilirubin. The contribution of extrahepatic tissues to bilirubin metabolism in humans [27]. During neonatal development, UGT1A1 gene expression was greater in the skin than the level in the liver. UGT1A1 expression and activity were even induced by UVB exposure of HaCaT cells, which photo-oxidizes tryptophan in the skin and produces 6-Formylindolo 3,2-b carbazole (FICZ), which is an agonist of AhR, which is a nuclear receptor seen in the expression of UGT1A1.

FICZ-treated HaCaT cells shown by quantitative study RT-PCR and qRT-PCR analysis shows better expression of the UGT1A1 in the HaCaT cells and also shows induction of Estradiol 3-*O*-Glucuronidation, which

is metabolized by UGT1A1 which shows positive result with increased microsomal activity.

It has been demonstrated that CYP1A1 does oxidize bilirubin, contributing to metabolizing bilirubin [28].

Clinical intervention to treat episodes of severe hyperbilirubinemia calls for extended phototherapy treatment or even blood transfusion. Phototherapy also has disadvantages. Increased insensible water loss, skin rashes, pyrexia, decreased maternal-infant interaction, and lack of visual sensory input are potential disadvantages of this treatment [29, 30]. Therefore, treatment of newborn infants with sunlight might be the ideal therapeutic method because it allows continuous breast-feeding.

CONCLUSION

UDP-Glucuronosyltransferase (UGT) 1A1, an enzyme of the glucuronidation pathway is the sole enzyme that can metabolize bilirubin. Due to the inadequate expression of UGT1A1 in the liver, infants will develop hyperbilirubinemia physiologically. Although phototherapy is effective in preventing jaundice, sunlight has also been suggested, to reduce serum bilirubin levels. Discussion is on the mRNA expression pattern of human UGT1A1 in human skin, human skin keratinocyte (HaCaT) cells, and skin of humanized UGT1 mice and multiple UGT1A isoforms, including UGT1A1, were expressed in human skin and HaCaT cells. The effect of UVB-exposed tryptophan on the HaCaT cells shows significant induction of UGT1A1 mRNA expression and the treatment of the HaCaT cells with 6-Formylindolo 3, 2-b Carbazole, which is one of the tryptophan derivatives formed by UVB, resulted in an induction of UGT1A1 mRNA and activity. The expression of UGT1A1 in neonates was greater in skin while that in adults was in liver. Treatment of humanized UGT1 mice with increased UGT1A1 expression and activity in the skin with reduction of serum bilirubin levels. Study suggests a protective role of UGT1A1 expression in the skin against neonatal hyperbilirubinemia. Sunlight allows possible alternative treatment for neonatal hyperbilirubinemia even allowing mother to breast their neonates.

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