Interindividual variability of glucuronidation of exogenous and endogenous compounds / 内因、外因性化合物のグルクロン酸抱合の個体差に関する研究

氏　　名	山中　洋幸
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論文審査委員（主査）	横井　毅（医学系研究科・教授）
論文審査委員（副査）	辻　彰（自然科学研究科・教授），宮本　謙一（医学部附属病院・教授），加藤　将夫（自然科学研究科・准教授），中島　美紀（医学系研究科・准教授）

Abstract

Glucuronidation which is catalyzed by UDP-glucuronosyltransferase (UGT) enzymes is a major elimination route of many xenobiotics and endobiotics, and thereby has important role in efficacy and toxicity of drug as well as hormone balance. The purpose of my study was to investigate the interindividual variability of glucuronidation of 4'-HPPH and thyroxine, as examples of exogenous and endogenous substrates of UGT, respectively. First, in vivo interindividual variability in 4'-HPPH glucuronidation was found to be 11 fold in 15 patients. The variability was not related with known polymorphic mutations of the *UGT1A1, UGT1A6,* and *UGT1A9* genes. By sequence analyses of *UGT1A* gene in a patient, a novel polymorphic allele, termed *UGT1A9*22*, was identified. Luciferase assay revealed that the allele increases the transcriptional activity. However, the allele was not related with the variability of 4'-HPPH glucuronidation in vivo. Second, in vitro interindividual variability in thyroxine glucuronidation was found to be 4 fold in 12 human liver microsomes. It was demonstrated that intestine and kidney would also contribute to the thyroxine glucuronidation. Third, it was demonstrated that 4'-HPPH and thyroxine glucuronidations catalyzed by multiple UGT1A isoforms were affected by the UGT-UGT interaction. The interindividual variability would result from the differences in the expression levels of each UGT isoform in livers among individuals, extrahepatic glucuronidation, genetic polymorphisms, and UGT-UGT interactions. These factors may intricately interact each other, complicating the understainging of interindividual variability of glucuronidations.

Dissertation abstract

Glucuronidation is a major route of elimination of many xenobiotics and endobiotics, and thereby has important role in efficacy and toxicity of drug as well as hormone balance. Glucuronidation is catalyzed by UDP-glucuronosyltransferases (UGTs). The purpose of my

study was to investigate the interindividual variability of glucuronidation of exogenous and endogenous compounds. The interindividual variability in glucuronidations of 4'-HPPH and thyroxine was evaluated as exogenous and endogenous substrates of UGT, respectively.

Interindividual variability of exogenous 4'-HPPH glucuronidation

Phenytoin, an anticonvulsant agent, is mainly excreted as 4'-HPPH *O*-glucuronide in humans. Phenytoin is metabolized to 4'-HPPH by CYP2C9 and CYP2C19, and 4'-HPPH is further metabolized to 4'-HPPH *O*-glucuronide by multiple UGTs of UGT1A1, UGT1A4, UGT1A6, and UGT1A9. 4'-HPPH may be bioactivated to a reactive metabolite that is associated with side effects such as gingival hyperplasia, somnolence, dry month, and general fatigue. Therefore, the glucuronidation is considered to be a detoxification pathway. In this study, the extent of interindividual variability in the urinary excretion levels of 4'-HPPH and its *O*-glucuronide was in related with genetic polymorphisms of *UGT1A* and *CYP2C*. 4'-HPPH and its glucuronide in urine samples from 15 patients to whom phenytoin was administered were measured by liquid chromatography-tandem mass spectrometry. When the molar ratio of 4'-HPPH *O*-glucuronide/4'-HPPH was calculated as an index of glucuronidation, a large interindividual variability (11 fold) was observed. Although 5 patients were heterozygotes of mutated alleles of *CYP2C9* or *CYP2C19* genes, no relationship with the interindividual difference in the total excretion of 4'-HPPH and its *O*-glucuronide was observed. The *UGT1A1*6, UGT1A1*28, UGT1A1*60* and *UGT1A6*2* alleles were found in 1, 3, 6, and 8 patients, respectively. There was no relationship between the genetic polymorphisms of *UGT1As* and the interindividual difference in the 4'-HPPH glucuronidation [1].

To elucidate new unknown polymorphic allele of UGTs that may affect the 4'-HPPH glucuronidation, all exons, exon-intron junctions, and the 5'-flanking region of the *UGT1A1, UGT1A4, UGT1A6,* and *UGT1A9* genes in a patient, who had extremely low 4'-HPPH glucuronosyltransferase activity were sequenced. As the result, one base insertion of thymidine in a promoter region of the *UGT1A9* gene resulting in A(T)$_{10}$AT was identified comparing with the reference sequence of AF297093 (A(T)$_9$AT). The allele was termed *UGT1A9*22*. The luciferase activity of the promoter construct containing the A(T)$_{10}$AT sequence was 2.6-fold higher than that of the construct containing the A(T)$_9$AT sequence. The mutant allele was expected to alter the UGT1A9 expression level. However, the mutant allele was not associated with the interindividual variability in 4'-HPPH glucuronidation in vivo in human [2]. It was reported that certain UGT isoform changes the enzyme activity of other UGT isoforms [3,4,5]. Furthermore, Kurkela et al [6] recently reported that the activity of mutant UGT was affected by wild type of other UGT isoforms. Therefore, in vivo glucuronidation may not reflect the changed activity of the enzyme by genetic polymorphisms which was evaluated in vitro.

4'-HPPH has a chiral center. (*S*)-4'-HPPH is a predominant form produced from phenytoin in humans, and (*R*)-4'-HPPH is an extremely toxic form with respect to gingival hyperplasia. When racemic 4'-HPPH was used as a substrate, human liver microsomes predominantly formed (*S*)-4'-HPPH *O*-glucuronide rather than (*R*)-4'-HPPH *O*-glucuronide. Among human UGT enzymes, UGT1A1 stereoselectively formed (*R*)-4'-HPPH *O*-glucuronide, whereas UGT1A9 and UGT2B15 stereoselectively formed (*S*)-4'-HPPH *O*-glucuronide from racemic 4'-HPPH. Using UGT1As double expression systems in HEK293 cells that previously established in our laboratory [3,4], the effects of UGT-UGT interaction on 4'-HPPH *O*-glucuronide formation were investigated. It was demonstrated that coexpression of UGT1A4 increased the Vmax values of (*S*)- and (*R*)-4'-HPPH *O*-glucuronide formation catalyzed by UGT1A1, but decreased the Vmax values of (*S*)- and (*R*)-4'-HPPH *O*-glucuronide formation catalyzed by UGT1A9. Coexpression of UGT1A6 increased the S_{50} values and decreased the Vmax values of (*S*)- and (*R*)-4'-HPPH glucuronide formation catalyzed by UGT1A1 and UGT1A9. However, the interaction did not alter the stereoselectivity [7]. In human tissues, the expression levels of each UGT isoform and relative ratio of them vary among individuals, indicating that the effects of the UGT-UGT interactions were different within the individuals. Thus, the UGT-UGT interaction would be one of factors causing the interindividual variability of glucuronidation.

Interindividual variability of endogenous thyroxine glucuronidation

Thyroxine is a major form of thyroid hormone secreted from thyroid gland, and is orally administered in hypothyroidism. Glucuronidation of thyroxine is a major metabolic pathway facilitating its excretion. The interindividual variability of thyroxine glucuronidation may be a casual factor affecting the plasma thyroxine concentration. In this study, the interindividual variability of thyroxine glucuronidation were evaluated, and human UGT isoforms involved in the activity were identified. Furthermore, extrahepatic glucuronidation were compared with hepatic glucuronidation. Eadie-Hofstee plots of thyroxine glucuronidation in human liver, jejunum, and kidney microsomes were monophasic. Human jejunum microsomes showed a lower Km value (24 μM) than human liver (86 μM) and kidney (53 μM) microsomes. Human kidney microsomes showed a lower Vmax value (23 pmol/min/mg) than human liver (133 pmol/min/mg) and jejunum (185 pmol/min/mg) microsomes. By scaling-up, the in vivo clearances in liver, intestine, and kidney were estimated to be 1440, 702, and 79 μl/min/kg of body weight, respectively. Therefore, thyroxine glucuronidation in extrahepatic tissue, especially in intestine, would contribute to the thyroxine glucuronidation in vivo. Using recombinant human UGT isoforms expressed in baculovirus-infected insect cells, UGT1A8 (109 pmol/min/unit (S)), UGT1A3 (92 pmol/min/unit (S)), and UGT1A10 (47 pmol/min/unit

(S)) showed high, and UGT1A1 (26 pmol/min/unit (S)) showed moderate thyroxine glucuronosyltransferase activity. The apparent Km value of recombinant UGT1A1 (105 μM) was similar to that of human liver microsomes. The thyroxine glucuronosyltransferase activity in microsomes from 12 human livers ranged from 23.7 to 84.8 pmol/min/mg of protein, representing 4 fold variability. It was significantly correlated with bilirubin *O*- ($r = 0.855, p < 0.001$) and estradiol 3-*O*- ($r = 0.827, p < 0.0001$) glucuronosyltransferase activities catalyzed by UGT1A1, indicating that the activity in human liver is mainly catalyzed by UGT1A1. Kinetic and inhibition analyses suggested that the thyroxine glucuronidation in human jejunum microsomes was mainly catalyzed by UGT1A8 and UGT1A10 and to a lesser extent UGT1A1, and the activity in human kidney microsomes was mainly catalyzed by UGT1A7, UGT1A9 and UGT1A10. The contribution of each UGT1A isoform would vary between human tissues, depending on the relative abundance of each isoform. When the effects of UGT-UGT interaction on thyroxine glucuronides were investigated, it was found that the coexpression of UGT1A4 and UGT1A6 differently affected the kinetics of the thyroxine glucuronide formation by UGT1A1 or UGT1A9. Thus, the interactions may affect the interindividual variability of thyroxine glucuronidation [8].

Conclusion

In this thesis, the interindividual variability of glucuronidation of exogenous and endogenous compounds was evaluated. The interindividual variability would result from the differences in the expression levels of each UGT isoform in livers among individuals, extrahepatic glucuronidation, genetic polymorphisms, and UGT-UGT interactions. These factors may intricately interact each other, complicating the understanding of interindividual variability of glucuronidations. Finally, the information described in this thesis could facilitate understanding about the interindividual variability in glucuronidation.

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学位論文審査結果の要旨

UDP-glucuronosyltransferase (UGT) によって触媒されるグルクロン酸抱合反応は生体における外因性および内因性の化合物の主要な排泄経路であり、薬物の効果や毒性、ホルモンバランスの調節に重要な役割を果たしている。本研究では、外因性および内因性化合物のグルクロン酸抱合反応の個体差について、外因性および内因性化合物として4'-HPPHおよびチロキシンを用いた。第一に、15名の被験者においてin vivoの4'-HPPHのグルクロン酸抱合反応に11倍の個体差が存在することを明らかにした。この個体差と*UGT*の既知の遺伝子多型との関連は認められなかったため、グルクロン酸抱合能の顕著に低い被験者の遺伝子を解析したところ、*UGT1A9*のpromoter領域に挿入変異を有する新規変異型*UGT1A9*22*を発見した。第二に、チロキシンのグルクロン酸抱合能に4倍の個体差を見出した。肝臓だけでなく、小腸や腎臓もチロキシンのグルクロン酸抱合を触媒し、それぞれ異なるUGT分子種が関与していることを示した。第三に、4'-HPPHおよびチロキシンのグルクロン酸抱合反応は、UGT-UGT蛋白相互作用により酵素活性が影響を受けることを明らかにした。以上、グルクロン酸抱合反応の個体差には、肝臓における各UGT分子種の発現量の個体差、肝外臓器におけるグルクロン酸抱合、遺伝子多型、UGT-UGT蛋白相互作用などの因子が複合して関与していることを明らかにした。本研究成果は、グルクロン酸抱合の個体差の理解のために有用な情報であり、博士（薬学）に値すると判定した。