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Effect of carbamazepine on expression of UDP-glucuronosyltransferase 1A6 and 1A7 in rat brain

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ABSTRACT

Because UDP-glucuronosyltransferase (Ugt) 1a6 and Ugt1a7 are highly expressed in the rat brain, changes in Ugt1a6 and Ugt1a7 expression may affect the pharmacokinetics of drugs and endogenous compounds in the brain. The present study aimed to elucidate the effect of carbamazepine (CBZ), a typical UGT inducer, on Ugt1a6 and Ugt1a7 expression in the rat brain. Sprague-Dawley rats were treated intraperitoneally for 7 d with CBZ (100 mg/kg/d). Ugt1a6 and Ugt1a7 mRNAs were induced by CBZ in the cerebellum, piriform cortex, and hippocampus (Ugt1a6: 3.1-, 2.4-, and 1.9-fold, respectively, Ugt1a7: 2.3-, 1.6-, and 3.1-fold, respectively); serotonin glucuronidation, which is catalyzed by Ugt1a6, was also increased by 2.8-, 1.7-, and 1.8-fold in these regions, respectively. The nuclear translocation of the constitutive androstane receptor was increased 1.4-fold in the cerebellum and piriform cortex, suggesting that brain Ugt1a6 and Ugt1a7 might be induced via the constitutive androstane receptor. However, the pregnane X receptor and nuclear factor erythroid 2-related factor 2 did not play decisive roles in the induction. Histone H3 lysine 9 acetylation, H3 lysine 4 pan-methylation, and H3 lysine 9 mono-methylation may not be required for the induction. This study clarified that CBZ affected Ugt1a6 and Ugt1a7 in the brain.

1. Introduction

UDP-glucuronosyltransferase (UGT) conjugates glucuronic acid to the parent compound and produces a more water-soluble metabolite, mainly in the liver. UGT1A is involved in the glucuronidation of central-acting drugs, including midazolam [1], propofol [2], and valproic acid [3], and it has been found in the brain [4,5]. Moreover, UGT1A has the potential to metabolize neurotransmitters such as serotonin [6] and dopamine [7]. Thus, changes in UGT1A expression in the brain may affect the brain concentrations of these UGT1A substrates, leading to the alteration of their pharmacological and physiological effects. UGT1A is induced by xenobiotics via nuclear receptors such as the pregnane X receptor (PXR), nuclear factor erythroid 2-related factor 2 (Nrf2), and constitutive androstane receptor (CAR) [8,9]. A typical inducer of UGT isoforms is the anti-epileptic agent carbamazepine (CBZ) [10]. CBZ is considered to activate PXR [11], which regulates the gene expression of Ugt1a6 and Ugt1a7 in rats [12]. These findings are consistent with a previous report that Ugt1a6 mRNA expression was induced by CBZ in the rat liver [13]. Because Ugt1a6 and Ugt1a7 mRNAs were detected at higher levels in the rat brain [14], CBZ may have the potential to alter the expression of Ugt1a6 and Ugt1a7 in the rat brain. A previous study reported that CBZ was metabolized by cytochrome P450 (CYP) to 2-hydroxy-CBZ (a reactive metabolite), which generates reactive oxygen species (ROS) in the liver [15]. Oxidative stress caused by ROS can result in cell death or cellular damage, but Nrf2 protects cells against oxidative stress through the induction of antioxidant enzymes [16]. It has been reported that the oxidative stress inducer paraquat alters the expression of Ugt1a6 and Ugt1a7 in rat astrocytes [17]. ROS generated by 2-hydroxy-CBZ can cause the induction of Ugt1a6 and Ugt1a7 via the Nrf2 signaling pathway in the brain. Moreover, CBZ enhances the nuclear translocation of CAR [18], leading to the transcriptional activation of the representative CAR-target gene CYP2B1 in the rat liver [19]. The expression levels of Ugt1a6 and Ugt1a7 mRNA were shown to increase following treatment with some CAR activators in the rat liver [20]. Because CAR mRNA has been detected in various areas of the rat brain [21], it is possible that Ugt1a6 and Ugt1a7 may be induced by CBZ via CAR activation in the rat brain.

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Recently, it was reported that changes in the histone modification-state were associated with the expression of the UGT isoforms [22,23]. Several covalent modifications on the N-terminal region of histone tails have many regulatory functions. In general, histone H3 lysine 9 (H3K9) acetylation and histone H3 lysine 4 (H3K4) pan-methylation are linked to activating gene expression [24], whereas H3K9 mono-methylation is associated with gene silencing [25]. It is hypothesized that CBZ can change the histone modification linked to the alteration of CYP3A4 in the HepG2 human hepatocellular carcinoma cell line [26,27]. The changes in the histone modification-state may be relevant to the induction of Ugt1a6 and Ugt1a7 by CBZ.

The purpose of the present study was to elucidate the effect of CBZ on the expression of Ugt1a6 and Ugt1a7 in the rat brain. Furthermore, we investigated the induction mechanisms, specifically those mediated by nuclear receptors and epigenetic regulation.

2. Materials and methods

2.1. Materials

Alamethicin and uridine 5′-diphosphoglucuronic acid trisodium salt were purchased from Sigma-Aldrich (St. Louis, MO). CBZ was obtained from Wako Pure Chemicals (Osaka, Japan). Serotonin β-D-glucuronide and serotonin-d4 β-D-glucuronide were purchased from Toronto Research Chemicals (Toronto, Canada). All other chemicals and solvents were of the highest grade commercially available.

2.2. Treatment of animals

The present study was approved by the Institution Animal Care and Use Committee of Meijo University. Eight-week-old male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) were treated intraperitoneally once daily for 7 d with 100 mg/kg CBZ (n = 5) or corn oil as a control (n = 5). In the present study, the dosage of CBZ was determined by considering prior reports [19], which stated 100 mg/kg exhibited the maximum induction rate of CYP in the rat liver. The cerebellum, frontal cortex, parietal cortex, piriform cortex, hippocampus, medulla oblongata, olfactory bulb, striatum, and thalamus were removed from the rat brains. The livers of the rats were also collected. These tissues were frozen in liquid nitrogen and stored at −80 °C.

2.3. RNA extraction and quantitative real-time polymerase chain reactions

Total RNA was isolated from nine brain regions and the liver using TRIzol reagent (Life Technologies, Carlsbad, CA), and the samples from five rats in each group were pooled. The complementary DNA was synthesized using ReverTra Ace qPCR Kits (TOYOBO, Osaka, Japan). The Ugt1a6, Ugt1a7, PXR, CYP3A23/3A1, CYP3A2, NAD(P)H quinone dehydrogenase-1 (NQO-1), and CYP2B1 mRNAs were quantified by real-time polymerase chain reaction with a Thermal Cycler Dice Real Time System obtained from TAKARA BIO (Shiga, Japan). Primers (Table 1) were commercially synthesized by FASMAC (Kanagawa, Japan). Amplification was performed as follows: denaturation for 30 s at 95 °C followed by 40 cycles of denaturation for 5 s at 95 °C and annealing and extension steps for 60 s at 60 °C. TaqMan Gene expression assays were performed to measure CYP3A2 mRNA expression using the primer set Rn0075646_m1 (Applied Biosystems, Foster City, CA). All data were normalized to β-actin.

2.4. Preparation of brain microsomes

Brain microsomes from nine regions were prepared as described previously [33], and all preparations were stored at −80 °C. Brain microsomes from 10 rats in each group were pooled. Protein concentrations were determined using Bio-Rad Protein Assay Kits (Bio-Rad, Hercules, CA).

2.5. Serotonin glucuronidation

Serotonin glucuronidation in the rat brain was determined according to a previously described method [33] with slight modifications. The concentrations of microsomal protein and serotonin were 0.2 mg/mL and 1 mM, respectively. The reaction mixture was incubated at 37 °C for 1 h. In a preliminary study, the incubation time and protein concentrations were found to be within the linear range.

2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis/western blot analysis

The cerebellum, piriform cortex, hippocampus, and olfactory bulb were removed from the rat brains 2 h after the last CBZ treatment (n = 4). The livers (n = 3) were also collected. The pooled nuclear and cytoplasmic proteins were prepared using NE-PER Nuclear and Cytoplasmic Extraction Kits (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. Protein concentrations were measured with Pierce 660 nm Protein Assay Kits (Thermo Fisher Scientific). To prepare histone extracts, all steps were carried out following a previously described method [34]. The extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and immunodetection was performed using the specific primary antibodies, which were rabbit anti-Nrf2 (ab31163,

Table 1
Primer sequences used in the present study.

Gene	Accession no.		Sequence	Reference
Ugt1a6	NM_057105	Sense	5′-GGGAGAATCCAAATACTACAGGAG-3′	[21]
		Anti-sense	5′-CAGCAAAGTGGTTGTTCCCAAAGG-3′	
Ugt1a7	NM_130407	Sense	5′-CAGACCCCGGTGACTATGACA-3′	[21]
		Anti-sense	5′-CAACGTGAAGTCTGTGCGTAACA-3′	
PXR	NM_052980	Sense	5′-CGGCTACCTGCGGTGTTT-3′	[28]
		Anti-sense	5′-CAACAGTGAGGCCTGCAGAA-3′	
CYP3A23/3A1	NM_013105.2	Sense	5′-TGGTCATGATTCCATCTTATGCTC-3′	[29]
		Anti-sense	5′-GGCGAAATTCCTCAGGCTCT-3′	
		Probe	5′-TCACCGTGATCCACAGCACTGGC-3′	
NQO-1	NM_017000.3	Sense	5′-TCAGTTCCCATTGTATTGGTTTGG-3′	[30]
		Anti-sense	5′-AAGCAAGGTCTTCTTATTCTGGAA-3′	
CYP2B1	NM_001134844.1	Sense	5′-ATGTTTGGTGGAGGAACTGCG-3′	[31]
		Anti-sense	5′-CTGGCTGGAGAATGAACTTAGGA-3′	
β-actin	NM_031144	Sense	5′-GTGACGTTGACATCCGTAAA-3′	[32]
		Anti-sense	5′-CTCAGGAGGAGCAATGATCT-3′	

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