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Research Article

The Effect of Streptozotocin and Alloxan on the mRNA Expression of Rat Hepatic Transporters *In Vivo*

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Abstract. The effect of streptozotocin (STZ) and alloxan (ALX) on the hepatic messenger RNA (mRNA) expression of four transporters (Mrp2, Mdr1, Oct1, and Oatp1) was studied in the present work. After the healthy male Wistar rats were individually treated by a single intraperitoneal injection of ALX monohydrate (150 mg/kg) or STZ (50 mg/kg), the hepatic mRNA expression levels of Mrp2, Mdr1, Oct1, and Oatp1 were detected by real-time quantitative PCR. The results indicated that the mRNA expression levels of the Mrp2, Mdr1, Oct1, and Oatp1 in ALX-induced diabetic rats, as well as the hepatic mRNA expression of Mdr1 and Oatp1 in STZ-induced diabetic rats, were significantly decreased as compared with the control. The inhibition of ALX and STZ on hepatic transporter expression suggested that alterations of drug transporters under diabetic condition can be responsible for reduced drug clearance.

KEY WORDS: alloxan; diabetic rats; streptozotocin; transporter.

INTRODUCTION

As the toxic glucose analogs, both alloxan (ALX) and streptozotocin (STZ) are the most prominent diabetogenic chemicals in diabetic experimental research. Although their cytotoxicity is achieved *via* different pathways, the mechanisms of beta cell-selective action through uptake *via* the glucose transporter 2 and beta cell death *via* necrosis are identical (1); reactive oxygen species (ROS) in the case of ALX and DNA alkylation in the case of STZ mediate the toxic action of these glucose analogs (1–3).

Drug transporters, as the membrane transporter protein expressed in many tissues, especially in liver, mediate the transport of exogenous or endogenous substances inside and outside the cell membrane (4). It contains efflux transporters including multi-drug resistance protein (Mdr) and multi-drug resistance-associated protein (Mrp) and uptake transporters including organic anion transporters (Oatps) and organic cation transporters (Octs) (5–7). Among them, Mrp2 and Mdr1 are the main efflux transporters, and Oct1 and Oatp1 are the main uptake transporters in liver. Since transporters play important roles in drug disposition and elimination, many studies have been focus on their change in kidney, liver, and intestine in normal states and in renal failure (7–10).

The liver is an important site for drug metabolism. Both drug metabolizing enzymes and uptake and efflux transporters are important determinants of drug metabolism and drug clearance by the liver (11,12). Under diabetic condition, the expression of metabolic enzymes and transporters in the liver is altered. It was reported that the hepatic CYP1A2, 2B1/2, 2E1, and 3A1 was upregulated in STZ- and ALX-induced diabetic rats (13,14). Besides these, the hepatic Mrp2 were downregulated, whereas the hepatic Mdr2 and Oatp2 were upregulated in STZ-induced diabetic rats (15,16). Until now, the effects of STZ and ALX on hepatic transporter expression have not yet been established. In the present work, the effect of STZ and ALX on rat hepatic messenger RNA (mRNA) expression of *Mrp2*, *Mdr1*, *Oct1*, and *Oatp1* were evaluated *in vivo*.

MATERIALS AND METHODS

Materials diethylpyrocarbonate (DEPC), ALX and STZ were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Trizol and the primers for *β-actin*, *Mdr1*, *Mrp2*, *Oatp1*, and *Oct1* were purchased from Invitrogen (Carlsbad, CA, USA). TransStart Green qPCR SuperMix and reverse transcription kits were obtained from TOYOBO Co., Ltd. (Japan). All of the other chemicals were analytical grade.

Induction of diabetic 8-week-old male Wistar rats (weighing 225–250 g, purchased from the provincial Disease Prevention and Control Center of Hubei, China) was maintained in SPF animal room at temperature 22±2°C, 60±5% humidity, and 12/12 h day/night cycle. The rats received standard laboratory chow and had free access to food and water. The diabetic rat model was built by a single intraperitoneal injection of ALX (150 mg/kg body weight) (17) or STZ (50 mg/kg body weight) (11) after an overnight fast. Four days

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after ALX injection or 7 days after STZ injection, the fasting blood sugar was estimated by glucometer and the rats with plasma glucose levels of ≥16.7 mmol/L were chosen in the following study. The ALX- and STZ-control rats were treated by injecting them with saline intraperitoneally after an overnight fast, and then bred for 4 and 7 days after saline administration, respectively. Five rats from each group were sacrificed under pentobarbital anesthesia. Liver tissues were harvested and immediately stored in liquid nitrogen until analysis. All procedures were approved by Ethic Committee of Hubei University and complied with health guidelines for the care and use of laboratory animals.

Analysis of mRNA expression by real-time quantitative RT-PCR total RNA was extracted from each liver sample using TRIzol reagent according to the manufacturer's protocol. The integrity of RNA samples were analyzed by formaldehyde-agarose gel electrophoresis with the visualization of 18S and 28S rRNA bands. The purity of RNA samples was analyzed by UV detection at 260/280 nm. One hundred micrograms of RNA from each liver sample was reverse transcribed to complementary DNA (cDNA) by RT-PCR using 1 μL of Primer Mix, 1 μL of RT Enzyme Mix, 4 μL of 5×RT buffer, and nuclease-free water in a final volume of 20 μL. The cDNA was amplified by the real-time fluorescence quantitative PCR for 43 cycles in the following conditions: denaturation at 94°C for 15 s, annealing at 55°C for 30 s and extension at 72°C for 30 s. The primer sequences of four target genes and *β-actin* (used as the load control) were depicted in Table I. Fold increase was calculated using the $2^{-\Delta\Delta Ct}$ relative expression method (12).

Statistical Analyses. The data are presented as the mean±standard deviation (SD). Significant differences were evaluated using Dunnett's test. Values of $p<0.05$ were considered statistically significant.

RESULTS

The Hepatic mRNA Expression of *Mdr1*, *Mrp2*, *Oatp1*, and *Oct1* in ALX-Induced Diabetic Rats

The real-time quantitative RT-PCR analysis was conducted to determine the effect of ALX treatment on the hepatic mRNA expression levels of *Mdr1*, *Mrp2*, *Oatp1*, and *Oct1*. The results are summarized in Table II and indicated that the hepatic mRNA expression levels of *Mdr1*, *Mrp2*, *Oatp1*, and *Oct1* were significantly decreased by 3.45-, 2.22-, 3.33-, and 4.35-fold in ALX-induced diabetic rats compared with the control.

Table I. The Primer Sequence for Real-Time Quantitative PCR

Gene	Primer sequence	GenBank accession no.
β-actin	F: CCA CAG TCC ATG CCA TCA CTG C R: CCA GGC GGC ATG TCA GAT CC	NM_031144
Mdr1	F: TCA GGG TTT GGA GTG GAA G R: CGC TCG CTG ACG AAG TAT G	NM_012623
Mrp2	F: CTG GTT GGA AAC TTG GTC R: TCA ACT GCC ACA ATG TTG GT	NM_012833
Oatp1	F: AGA GGG TAG ATT GTT TTC C R: TGT GTT CGG TTC TCC ATA	NM_017111
Oct1	F: ACA GAA GAA CGG GAA GGT G R: AGA AGT CCA GGT AGA GGT T	NM_012697

Table II. The Effect of ALX on the mRNA Expression of *Mdr1*, *Mrp2*, *Oatp1*, and *Oct1* in Rat Liver

	Fold responses	
Gene	Control	ALX
Mdr1	1.00±0.45	0.29±0.34**
Mrp2	1.00±0.42	0.45±0.59*
Oatp1	1.00±0.45	0.30±0.40**
Oct1	1.00±0.39	0.23±0.48**

$n=5$
*$p<0.05$; ** $p<0.01$ (*vs* the control)

The Hepatic mRNA Expression of *Mdr1*, *Mrp2*, *Oatp1*, and *Oct1* in STZ-Induced Diabetic Rats

After STZ treatment, the hepatic mRNA expression levels of *Mdr1* and *Oatp1* were determined by real-time quantitative RT-PCR analysis, and the results are summarized in Table III. The hepatic mRNA expression levels of *Mdr1* and *Oatp1* were significantly decreased by 1.59- and 2.04-fold in STZ-induced diabetic rats as compared with the control. Although STZ treatment resulted in a slight increase for *Oct1* mRNA expression and a slight decrease for *Mrp2* mRNA expression, there was no statistical significance as compared with the control.

DISCUSSION

Hepatobiliary transport systems are important for bile salt homeostasis (18,19). Bile salts and organic anions are taken up into the liver *via* basolateral sodium taurocholate co-transporting peptide and OATPs and effluxed into bile at the canalicular domain of hepatocytes *via* bile salt export pump and Mrp2 (20). Diabetes mellitus is associated with the change of transporter expression in liver. Increased biliary bile salt and phospholipid output rates have been described in STZ- or ALX-induced diabetic rats (21,22). The hepatic Mrp2 were downregulated, whereas the hepatic *Mdr2* and *Oatp2*

Table III. The Effect of STZ on the Hepatic mRNA Expression of *Mdr1*, *Mrp2*, *Oatp1*, and *Oct1*

	Fold responses	
Gene	Control	STZ
Mdr1	1.00±0.17	0.63±0.39*
Mrp2	1.00±0.22	0.66±0.66
Oatp1	1.00±0.31	0.49±0.73*
Oct1	1.00±0.10	1.16±0.19

$n=5$
*$p<0.05$ (*vs* the control)

were upregulated in STZ-induced diabetic rats (15,16). Induction of Mdr2 expression and biliary phospholipid secretion was responsible for the enhanced capacity of biliary bile salt secretion in STZ-induced diabetic rats (16). And the reduced concentration of glutathione (the substrate of Mrp2) in the diabetes might decrease *Mrp2* mRNA expressions (15).

In the present work, the significant downregulation of the hepatic mRNA expression of *Mdr1*, *Mrp2*, *Oatp1*, and *Oct1* in ALX-induced diabetic rats, as well as the hepatic mRNA expression of *Mdr1* and *Oatp1* in STZ-induced diabetic rats, was observed as compared with their respective control. And the inhibition of ALX on the tested transporters was greater than that of STZ. It is known that Mrp2 (human) or Mrp2 (rat) is responsible for the export of numerous endogenous and xenobiotic compounds including taurocholate, methotrexate, glutathione, and statins (23,24). Mdr1 (human) or Mdr1 (rat) mediates multi-drug resistance by enhancing the active efflux of a broad range of therapeutic drugs including (*e.g.*, vinca alkaloids, berberine, anthracyclines, cyclosporin A, digoxin, and glucocorticoids) from cells (25). Oatp1 is an important member of OATPs superfamily that mediate the sodium-independent uptake of a diverse range of amphiphilic organic compounds including bile acids, steroid conjugates, thyroid hormones, anionic peptides, numerous drugs, and other xenobiotic substances in liver (26). Oct1 (human) is responsible for the hepatic uptake of relatively hydrophilic, low molecular mass organic cations (*e.g.*, tetraethylammonium, *N*-methylpyridinium, metformin, and oxaliplatin) (27). The changes in the mRNA expression of the tested four transporters suggested that STZ or ALX treatment can affect the pharmacokinetic, safety, and efficacy profiles of the substrate drugs of the tested four transporters in some extent.

However, the underlying mechanisms of the alteration in the mRNA expression of the tested transporters are not well characterized. To clarify these phenomena, the analysis for the protein expression and activity of these transporters are necessary in future study.

CONCLUSIONS

The hepatic uptake/efflux transporters play an important role in hepatic toxicity and adverse drug-drug interactions. Our results indicated that the mRNA expression levels of the *Mrp2*, *Mdr1*, *Oct1*, and *Oatp1* in ALX-induced diabetic rats, as well as the hepatic mRNA expression of *Mdr1* and *Oatp1* in STZ-induced diabetic rats, were significantly decreased as compared with their respective control. The inhibition of ALX and STZ on hepatic transporter expression suggested that alterations of drug transporters under diabetic condition should affect the pharmacokinetic, safety, and efficacy profiles of the substrate drugs of the tested four transporters in some extent.

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