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Downregulation of organic cation transporter 1 and breast cancer resistance protein with the induction of Pregnane X receptor in rat kidney impaired by doxorubicin

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Transporters expressed in the kidney play an important role in the excretion of endogenous substances and chemical drugs. The Pregnane X receptor (PXR) has been reported to be involved in regulating the expression of numerous transporters. In the present study, we examined the alteration in expression level of PXR, organic cation transporter 1 (OCT1) and breast cancer resistance protein (BCRP) in renal cell lines of rat origin and the kidney of rats when damaged by doxorubicin (DOX). The expression level of PXR in renal tubular epithelium NRK-52E cells was significantly increased by DOX at a concentration confirmed to cause cellular damage. The expression levels of OCT1 and BCRP were significantly lower in the DOX-treated cells than in the untreated cells. In model rats with DOX-induced nephrotoxicity, the alterations in renal expression of PXR, OCT1 and BCRP were similar to those in NRK-52E cells, although there was a difference in the degree of the changes. Therefore, the downregulation of OCT1 and BCRP may occur in response to PXR induction in the kidney impaired by DOX.

1. Introduction

Most cancer patients receive a variety of medicines to prevent complications and alleviate unpleasant symptoms related to the disease and its treatment. In multidrug regimens, drug-drug interaction is regarded as one of the important risk factors affecting the therapeutic outcome. Doxorubicin (DOX), an anthracycline derivative, is a widely used in chemotherapy of cancer. DOX alone or in combination with other chemotherapies is a common first-line therapy for numerous malignant tumors, including leukemia, and breast, ovarian, bladder, and lung cancers. Although it is clinically effective, severe and extensive organ toxicity is common during DOX treatment. In addition to cardiotoxicity, renal abnormalities have been reported in patients receiving DOX (Waga et al. 2010). The pharmacokinetics of therapeutic drugs excreted mainly *via* the kidneys are likely to change in patients with renal impairment induced by DOX. As structurally large or charged chemical drugs are susceptible to elimination from systemic circulation by transporter-mediated renal secretion (George et al. 2017), it is of practical importance to evaluate alterations in expression levels of tubular transporters in kidneys exposed to DOX.

The Pregnane X receptor (PXR) is known to be a key regulator of numerous transporters (Teng et al. 2008) and is expressed in the kidney (Luan et al. 2019). It was previously reported that several transcription modulators, such as p300 and FOXO1, increased the expression of PXR in several cell types (Pasquel et al. 2016). Exposure of cells, including renal cells, to DOX leads to the activation of p300 and FOXO1, and multiple-organ disorders develop through the induction of apoptosis and suppression of the cell cycle (Jain et al. 2012; Kavazis et al. 2014). Expression of organic cation transporter 1 (OCT1) and breast cancer resistance protein (BCRP) is increased in PXR knock-out mice (Gahir et al. 2017). Considering these

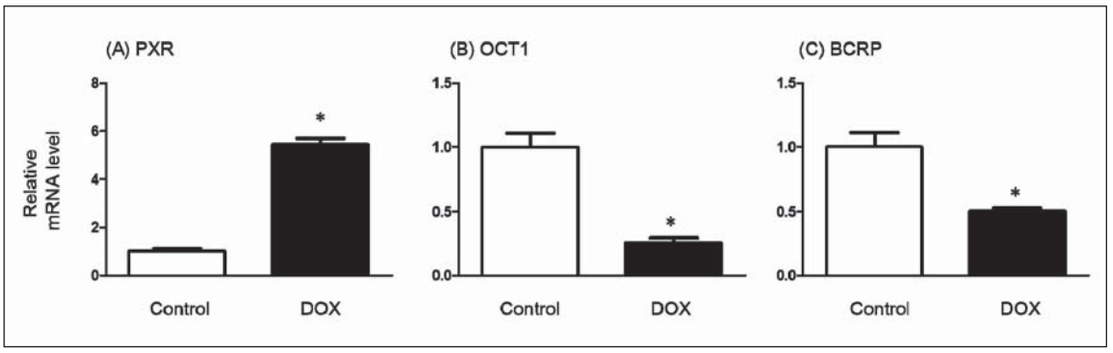

Fig. 1: Effects of DOX exposure on the expression of PXR, OCT1, and BCRP mRNA in NRK-52E cells. Data were normalized to GAPDH, which was used as an internal control. Results are shown as means±SD from three independent experiments. * Significantly different from the mean of the control group (p<0.05, Student's unpaired t-test).

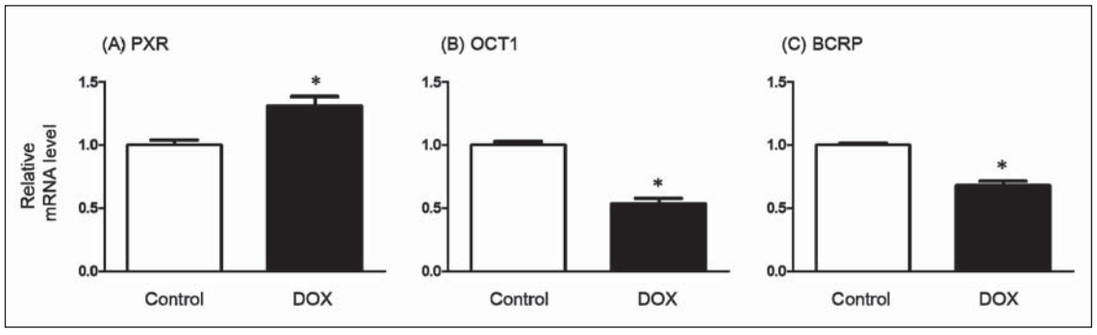

Fig. 2: Effects of DOX exposure on the expression of PXR, OCT1, and BCRP mRNA in rat kidneys. Data were normalized to GAPDH, which was used as an internal control. Results are shown as means ± SD from three independent experiments. *: Significantly different from the mean of the control group (p<0.05, Student's unpaired t-test).

findings, the expression of OCT1 and BCRP in the kidney may be suppressed by DOX. The aim of the present study was to examine using *in vitro* and *in vivo* analyses whether the expression of PXR, OCT1, and BCRP is subject to pre-transcriptional modification in rat-derived kidney cell lines and rat kidneys damaged by DOX.

2. Investigations, results and discussion

The concentration dependence of the cytocidal activity of DOX in NRK-52E cells was confirmed, with cell death occurring even in the presence of 1 mM DOX (data not shown). When NRK-52E cells were exposed to 1 mM DOX, which exhibited minimum toxicity, the expression of PXR was significantly increased, and correspondingly, the expression of OCT1 and BCRP was significantly reduced (Fig. 1). Next, we conducted an *in vivo* study using rats in order to clarify whether these effects were similar in the animal model. The increase in PXR expression in the kidneys was observed in rats administered DOX (15 mg/kg; i.p.), and correspondingly, the expression of OCT1 and BCRP was significantly reduced (Fig. 2). We previously demonstrated renal impairment following the administration of DOX (15 mg/kg; i.p.) to rats (Nagai et al. 2018). Based on the similarity in the changes in the expression of PXR to those in OCT1 and BCRP after DOX exposure between NRK-52E cells and the rat kidney, this study suggested that the renal expression levels of OCT1 and BCRP were reduced due to PXR induction by DOX treatment both *in vitro* and *in vivo*.

The pharmacokinetics of many drugs may be affected by the changes in OCT1 and BCRP expression in patients with renal damage caused by DOX. The OCT family, including OCT1, is abundantly expressed in the proximal tubules of the kidney and is involved in the elimination of basic drugs. As antitumor agents, such as cisplatin and sorafenib, used in combination with DOX serve as substrates of OCT1 (Lin et al. 2013; Herraez et al. 2013), the reduction in OCT1 expression is considered to lead to adverse events associated with the increase in the blood levels of these drugs. In addition to antineoplastic drugs, the analgesic morphine, and the antiemetic drug ondansetron are also known to be substrates of OCT1 (Tzvetkov et al. 2012, 2013). The decrease in OCT1 expression delays renal excretion of these concomitant drugs, which may result in overdose. BCRP functions in the excretion of numerous drugs, including antineoplastic agents, by utilizing ATP energy in the renal tubules. A previous study demonstrated that BCRP plays a role in the pharmacokinetics of antineoplastic agents such as methotrexate, irinotecan, imatinib, sunitinib (Mao and Unadkat 2015); therefore, early detection of the adverse events responsible for these drugs is required for patients with renal impairment caused by DOX. According to a previous study, chronic kidney disease is associated with the development of hyperuricemia (Takae et al. 2016). As uric acid was reported to be eliminated *via* BCRP in the renal tubules (Mao and Unadkat 2015), reduced expression of BCRP may be one of the risk factors for the increased incidence of hyperuricemia during renal injury. Therefore, it is of clinical importance to monitor the serum uric acid concentration to prevent gout during DOX treatment.

In conclusion, the present study demonstrated that the renal expression of OCT1 and BCRP was reduced through PXR induction when the kidney was damaged by DOX. Our findings will be of great help in promoting the correct use of anthracyclines for cancer chemotherapy.

3. Experimental

3.1. Chemicals

DOX hydrochloride was obtained from Meiji Seika Kaisha (Tokyo, Japan). Rat renal tubular epithelial cells (NRK-52 E cells) were purchased from the American Type Culture Collection (Manassas, VA, USA). All other reagents were of commercial or analytical grade requiring no further purification.

3.2. Cell culture

NRK-52E cells were maintained in Dulbecco's modified Eagle's MEM (Fujifilm Wako Pure Chemical Co., Osaka, Japan) containing 10% fetal bovine serum (Biowest USA, Riverside, MO, USA) at 37°C under a humidified atmosphere of 5% CO_2 in air.

3.3. Cell viability

DOX was dissolved in culture medium to a final concentration of 1 mM or 2 mM. Cell viability was evaluated using the MTT assay 24 hr after NRK-52E cells were treated with DOX.

3.4. Animal care

Male Sprague-Dawley rats, aged seven weeks, were obtained from Japan SLC, Inc. (Hamamatsu, Japan). Rats were acclimatized for at least four days before assignment to their experimental groups, and were housed in a clean room maintained at 23±2 °C, with a relative humidity of 55±10% and 12-h light/dark cycle. They were allowed free access to regular animal diet and tap water.

Table: Forward and reverse oligonucleotide primer sequences used for real-time PCR

	Forward	Reverse
PXR	TCCACTGCATGCTGAAGAAG	AACCTGTGTGCAGGATAGGG
OCT1	CTGCCTACCTTCCTCTTC	TTGCTCCATTATCCTGACA
BCRP	CAATGGGATCATGAAACCTG	GAGGCTGATGAATGGAGAA
GAPDH	TTCAACGGCACAGTCAAG	TACTCAGCACCAGCATCA

3.5. Real-time quantitative PCR

In the *in vitro* analysis, NRK-52E cells were treated with 1 mM DOX and total RNA was extracted from the cell lines by ISOGEN solution (Fujifilm Wako Pure Chemical Co.) 24 h later, followed by purification using a GenElute Mammalian Total RNA Miniprep kit (Sigma-Aldrich Co., St. Louis, MO, USA). The RNA was reverse-transcribed into cDNA by Oligo-T priming and Moloney murine leukemia virus reverse transcriptase (GE Healthcare, Seattle, WA, USA). The expression levels of targeted genes were analyzed by real-time quantitative PCR with MyiQ2 (Bio-Rad Lab., Inc., Berkeley, CA, USA) using SYBR green as the fluorescent dye (TOYOBO Co., Ltd.,

Osaka, Japan). cDNA was PCR-amplified at 95 °C for 10 s, 53 °C for 10 s, and 72 °C for 30 s. In the initial experiments, melting curve analysis was performed to monitor PCR product purity. The relative mRNA expression of targeted genes was calculated using the comparative threshold cycle number for each sample. To adjust for variations in the amount of DNA, the gene expression of the target sequence was normalized to the expression of an endogenous control, GAPDH. The synthetic oligonucleotide primers (Hokkaido System Science Co., Ltd., Sapporo, Japan) used to investigate the expression levels of targeted genes were designed by Beacon Designer 8 (Bio-Rad Lab., Inc.) and are listed in the Table. mRNA levels were quantified based on standard curves. Results were expressed relative to control values, which were an arbitrary value of 1.

In the *in vivo* analysis, rats were assigned to two treatment groups, which were designated as DOX-treated and control group. As in our previous report (Nagai et al. 2018), in the DOX-treated group, 15 mg/kg of DOX dissolved in physiological saline was intraperitoneally administered to rats on the first day. Control rats received physiological saline only at the same time. Total RNA was extracted from renal tissues on the 5th day. The real-time PCR conditions were the same as those adopted to measure the expression levels of target genes in NRK-52E cells. Experimental protocols and animal care methods were approved by the Animal Experiment Committee of Osaka Ohtani University.

3.6. Statistical analysis

The data are expressed as means±S.D. Differences between the means of two groups were compared using the Student's unpaired *t*-test. Differences with a *p*-value of 0.05 or less were considered significant.

Conflicts of interest: None declared.

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