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**EFFECTS OF ALTERATIONS IN CAR ON BILIRUBIN
DETOXIFICATION IN MOUSE COLLAGEN-INDUCED
ARTHRITIS**

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Running title page

EFFECTS OF CAR ON BILIRUBIN DETOXIFICATION IN ARTHRITIS

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Abbreviations: CAR, constitutive androstane receptor; PXR, pregnane X receptor; CYP,
cytochrome P450; CIA, collagen-induced arthritis; PB, phenobarbital; TCPOBOP,
1,4-bis [2-(3,5-dichloropyridyloxy)] benzene; OATP, organic anion transporter peptide;
GST, glutathione S-transferase; UGT, UDP-glucuronosyltransferase; MRP2, multidrug
resistance-associated protein 2; CCRP, cytoplasmic CAR retention protein

Abstract

Nuclear receptors such as constitutive androstane receptor (CAR) and pregnane X receptor (PXR) regulate the transcription of cytochrome P450s (CYPs) and transporters. We investigated whether quantitative and functional changes in CAR and PXR could affect bilirubin detoxification in chronic arthritis. The CAR mRNA level was significantly decreased in the liver of mice with collagen-induced arthritis (CIA) compared to control mice. In normal mice treated with CAR agonists, relatively rapid elimination of bilirubin was observed after its intravenous injection. Next, we investigated the effects of CAR on bilirubin-detoxifying enzymes and transporters in arthritis. The mRNA levels of organic anion transporter peptide (OATP) 2, glutathione S-transferase (GST) A1 and GSTA2 were decreased in CIA mice, whereas the mRNA levels of OATP4, UDP-glucuronosyltransferase (UGT) 1A1 and multidrug resistance-associated protein 2 (MRP2) remained unchanged. The protein levels and transport activities of OATP2 were also decreased in CIA mice. Furthermore, the CIA mice actually exhibited retarded elimination of bilirubin after its intravenous injection. These results indicate that alterations to CAR during arthritis affect the elimination of bilirubin due to changes in multiple bilirubin-detoxifying enzymes and transporters.

The nuclear receptors constitutive androstane receptor (CAR, NR1I3) and pregnane X receptor (PXR, NR1I2) are involved in the primary response to xenobiotics and endogenous toxins. Specifically, these receptors respond to ligands by activating the expressions of genes encoding enzymes involved in phase I (functionalization reactions) and phase II (conjugation reactions) metabolism and transporters (Kliwer et al., 1998; Honkakoski et al., 1998; Waxman et al., 1999). CAR is normally sequestered in the cytoplasm of untreated liver cells and becomes translocated to the nucleus after exposure to phenobarbital (PB) or PB-like chemicals. Cytoplasmic CAR retention protein (CCRP) has been shown to maintain the cytoplasmic localization of CAR by forming a complex with CAR and hsp90 (Squires et al., 2004; Kobayashi et al., 2003). Although much is known about the roles these two receptors play in coregulating cytochrome P450 (CYP) gene expression, comparatively little is known about their roles in coregulating transporter gene expression in vivo.

It has been reported that CAR and PXR also regulate the expressions of bilirubin-detoxifying enzymes and transporters (Huang et al., 2003; Wagner et al., 2005). Bilirubin is an oxidative end product of heme catabolism, and is usually removed from the body into the bile through the actions of liver cells. Detoxification of bilirubin is a

multistep process (Jansen et al., 1995; Kamisako et al., 2000). In the circulation, bilirubin is bound noncovalently to serum albumin. Free bilirubin enters the liver via the sinusoidal surface of hepatocytes by facilitated diffusion, which is mediated by organic anion transporter peptide (OATP) 4/Slco1b2 and OATP2/Slco1a4. Although this transport is bidirectional, binding to cytosolic ligandin, a homodimer or heterodimer of glutathione S-transferase (GST) A1 and GSTA2, reduces the efflux and increases the net uptake. Bilirubin is glucuronized in the endoplasmic reticulum by the specific bilirubin UDP-glucuronosyltransferase (UGT) 1A1. The final step in the clearance is secretion of bilirubin diglucuronide across the canalicular membrane into the bile, which is mediated by multidrug resistance-associated protein 2 (MRP2, Abcc2). Several studies have shown that UGT1A1 is a target gene for CAR and PXR. For example, treatment with CAR agonists, such as PB and 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP), increased the hepatic expressions of UGT1A1, GSTA1, GSTA2, MRP2 and OATP4/SLC21A6, and these inductions were absent in CAR-null mice (Sugatani et al., 2001; Huang et al., 2004). As stated above, CAR regulates the hepatic expression of each component of the bilirubin clearance pathway, while PXR has been shown to play both positive and negative roles in regulating bilirubin homeostasis (Saini et al., 2005).

Under several pathological conditions, it is possible that upregulation or downregulation of CAR and PXR could quantitatively modify bilirubin detoxification. However, few reports are available regarding the relationships between alterations in CAR or PXR and bilirubin detoxification under pathological conditions, despite the need to clarify the changes in their physiological functions. In the present study, we investigated bilirubin detoxification under chronic arthritis conditions, similar to rheumatoid arthritis, since this information is important for assessing the pharmacokinetics of various drugs due to changes in the protein binding and drug metabolism (Piquette-Miller et al., 1992; Piquette-Miller et al., 1995; Pollock et al., 1989; Walker et al., 1986). Specifically, we determined the mRNA levels of CAR, PXR and CCRP in the liver of mice with collagen-induced arthritis (CIA). We found altered expressions of bilirubin-detoxifying enzymes and transporters, as well as decreases in the mRNA levels of OATP2, GSTA1 and GSTA2 in CIA mice. Furthermore, elimination of bilirubin was retarded in CIA mice by the decreased levels of bilirubin-detoxifying factors, indicating that alterations in CAR during arthritis affected the elimination of bilirubin.

Methods

Animals. Eight-week-old male DBA/1J mice were purchased from Japan SLC Co. (Shizuoka, Japan) and maintained under conventional housing conditions. CIA was induced in the mice by intradermal immunization with 100 μ g of bovine tracheal cartilage type II collagen (Sigma, St. Louis, MO) emulsified in Freund's incomplete adjuvant (Difco, Detroit, MI) in Bayol F oil (Wako Pure Chemical Industry, Osaka, Japan) under ether anesthesia. The immunization was carried out via two injections at the dorsal skin at 0 and 21 days. Control mice were treated with Bayol F oil alone. To follow the development of arthritis, hindpaw swelling of the left ankle was measured using a plethysmometer. Hindpaw swelling was calculated using the following formula: percentage swelling = $(V - V_0) / V_0 \times 100$, where V_0 and V represent the hindpaw volumes before and after adjuvant treatment, respectively. We used the CIA mice at 42 days after immunization. The experiments were approved by the Committee for the Care and Use of Laboratory Animals at Kinki University School of Pharmaceutical Science.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). For RT-PCR

analyses, we collected the livers from control and CIA mice. Total RNA was extracted from approximately 100 mg of each liver using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Following RNase-free DNase I treatment (TaKaRa, Shiga, Japan), 500 ng of RNA, as evaluated by the UV absorption at 260 nm, was reverse-transcribed to complementary DNA (cDNA) using an RNA PCR Kit (AMV) Ver. 3.0 (TaKaRa) according to the manufacturer's instructions. The samples were then incubated at 94°C for 2 min, and amplified by 25 cycles of 94°C for 30 s, 52°C (CAR, PXR, CCRP and 28s rRNA) or 55°C (UGT1A1, MRP2, OATP4, OATP2, GSTA1 and GSTA2) for 30 s and 72°C for 1 min. The amount of each cDNA was analyzed using the Scion Image Beta 4.03 software (Scion Corporation, Frederick, MD). We further confirmed that the cDNA level for each target gene could not be saturated in 25 cycles of amplification to estimate mRNA levels correctly in RT-PCR method. The amount of 28S rRNA in each sample was also measured for normalization. For the PCR reactions, we used the following oligonucleotide sequences: CAR: 5'-GGAGGACCAGATCTCCCTTC-3' and 5'-ATTTTCATTGCCACTCCCAAG-3'; PXR: 5'-CCCATCAACGTAGAGGAGGA-3' and 5'-GGGGGTTGGTAGTTCAGAT-3'; CCRP: 5'-ATAAAAATGCCAGGCACAG-3' and 5'-CTGGGTATCGACCAAGCATT-3';

UGT1A1: 5'-CCTTGGATGTGATTGGCTTCC-3' and
5'-CCCTTTCCCCAAAGCATT-3'; MRP2: 5'-CCCTGGAATTGGCTCATCTCA-3'
and 5'-ATTTTCGAAGCACGGCCCT-3'; OATP4:
5'-CCATCTTCCATTTCGGTTTG-3' and 5'-ACTCCCAATGCCCATGATGAA-3';
OATP2: 5'-AATTCGGTATCCCCACGTCTG-3' and
5'-AGGCCTGTGCAGTTTTGTTC-3'; GSTA1:
5'-CAGCCTCCCCAATGTGAAGAA-3' and 5'-TGGCTCCATCAATGCAGCTT-3';
GSTA2: 5'-AGCTTGATGCCAGCCTTCTGA-3' and
TTTCTCTGGCTGCCAGGATGT-3'; and 28S rRNA:
5'-CGGCTCTTCCATCATTGTG-3' and 5'-CCTGTCTCACGACGGTCTAA-3'.

Measurement of Bilirubin Clearance. Mice were treated with intraperitoneal injections of the clinically used CAR ligand PB (50 mg/kg body weight, dissolved in DMSO; Fujinaga Pharm Co., Tokyo, Japan) or the specific, more potent, rodent CAR ligand TCPOBOP (3 mg/kg body weight, dissolved in corn oil; Sigma) over a period of 3 days. Control, CIA, PB-treated and TCPOBOP-treated mice were each injected via the tail vein with bilirubin (10 mg/kg body weight; Wako, Tokyo, Japan) dissolved in an isotonic solution containing 0.5 g of Na₂CO₃ and 0.52 g of NaCl per 100 mL. After 10

or 60 min, serum samples were collected and the total, direct and indirect bilirubin levels were determined by Osaka Kessei Research Laboratories (Osaka, Japan).

Measurement of GSH Levels in Livers. To clarify the status of GSH, the GSH levels in the livers were measured by using Glutathione Assay Kit (Cayman Chemical Company, Ann Arbor, MI). The values of GSH levels represented total GSH levels (μM) in liver homogenates.

Western Blot Analysis for OATP2. Mice were euthanized and the livers were homogenized individually in PBS containing 250 mM sucrose and 1%(v/v) Sigma protease inhibitor cocktail (Sigma Aldrich) at 4 °C. The homogenates were centrifuged at $1500 \times g$ for 30 min. An aliquot of PBS was added to the resultant pellets and centrifugation was repeated. The protein concentrations of the supernatants obtained after two centrifugations, which were used as protein samples, were determined using a BCATM Protein Assay Kit (PIERCE, Rockford, IL). Fifty micrograms of proteins was diluted with loading buffer, denatured at 95 °C for 3 min, and resolved by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10-20% polyacrylamide) and transferred to a polyvinylidene fluoride

(PVDF) membrane (Immobilon-P; Millipore Corp., Bedford, MA) by semidry blotting with Transblot SD (Bio-Rad, Hercules, CA). OATP-2 protein was detected by Western Breeze Chemiluminescent Detection System, anti-Goat (Invitrogen). The primary polyclonal mouse antibody (M-13) (Santa Cruz Biotechnology, Inc. CA) (1:1000) was used. Protein bands were visualized by exposure to BioMax XAR Film (KODAK, Cedex, France).

Preparation of Mouse Hepatocytes. Mouse hepatocytes were isolated using a two-step collagenase perfusion method (Nishikawa et al., 1998). Mouse were anesthetized by inhalation of ether. The liver was perfused in situ with calcium-free Hanks' buffer containing EGTA (0.5 mM) for 10 min at 37°C followed by perfusion of Hanks' buffer with collagenase type IV (0.7 U/mL) and calcium chloride (4 mM) for 10 min. The hepatocytes were released from the digested liver, and the suspension was filtered through a nylon mesh with 100- μ m pores and then was centrifuged. The obtained cell pellet was resuspended in Krebs-Henseleit buffer (KHB). KHB consisted of 118 mM NaCl, 23.8 mM NaHCO₃, 4.8 mM KCl, 1.0 mM KH₂PO₄, 1.2 mM MgSO₄, 12.5 mM HEPES, 5.0 mM glucose, and 1.5 mM CaCl₂ adjusted to pH 7.4. The number of viable cells was determined by trypan blue staining.

Determination of Uptake Activities of Estrone 3-sulfate in Hepatocytes. The uptake assay in hepatocytes was carried out by the using of 1 μ M Estrone 3-sulfate (Sigma), specific OATP2 substrate, containing [3 H]estrone 3-sulfate (Perkin Elmer, Wellesley, MA). The hepatocytes were quickly suspended in 10 mL of ice-cold KHB. The cells were resuspended in the same buffer to give a cell density of 2.0×10^6 viable cells/ml for the uptake study. Prior to the uptake studies, the cell suspensions were prewarmed in an incubator at 37°C for 3 min. The uptake studies were initiated by adding an equal volume of buffer containing labeled and unlabeled substrates to the cell suspension. After incubation at 37°C and 4°C for 0.5, 2 or 5 min, the reaction was terminated by separating the cells from the substrate solution. For this purpose, an aliquot of 100 μ L of incubation mixture was collected and placed in a centrifuge tube (500 μ L) containing 50 μ L of 2 N NaOH under a layer of 100 μ L of oil (density, 1.015, a mixture of silicone oil and mineral oil; Sigma-Aldrich), and subsequently the sample tube was centrifuged for 30 s using a tabletop centrifuge (10,000 g, KR-1500, Kubota, Tokyo, Japan). During this process, hepatocytes passed through the oil layer into the alkaline solution. After an overnight incubation in alkali to dissolve the hepatocytes, each compartment of the centrifuge tube was transferred to a scintillation vial. The

compartment containing the dissolved cells was neutralized with 50 μ L of 2 N HCl, mixed with scintillation cocktail, and the radioactivity was measured in a liquid scintillation counter (Tri-Carb 2800TR, Perkin Elmer, Wellesley, MA) after 5 mL of scintillation fluid (Clear-sol I; Nacalai Tesque, Kyoto, Japan) to the scintillation vials.

Statistical Analysis. Significant differences between mean values of the gene expression levels were estimated using Student's unpaired t-test after ANOVA.

Results

In this study, we used CIA mice to investigate the effects of CAR or PXR on the expressions of bilirubin-detoxifying enzymes and transporters in chronic arthritis. To confirm the induction of CIA, hindpaw swelling of the left ankle was measured up to 42 days. Fig. 1 shows the body weights and foot volumes of the immunized mice. Foot volume was significantly increased from 10 days after immunization, whereas little difference in the body weight between control and CIA mice was observed.

The mRNA levels of CAR, PXR and CCRP in the livers of control and CIA mice are shown in Fig. 2. The CAR mRNA level in CIA mice was significantly decreased to about 55% of the level in control mice. The mRNA levels of PXR and CCRP exhibited little alterations between control and CIA mice. These results indicate that the inflammatory condition in CIA mice may affect the expressions of enzymes and transporters regulated by CAR.

We assumed that the suppressed levels of CAR would decrease bilirubin clearance due to downregulation of bilirubin-detoxifying enzymes and transporters. To clarify

whether CAR participated in the regulation of bilirubin clearance, we examined bilirubin elimination after its intravenous injection into mice with or without treatment with the potent CAR agonists PB and TCPOBOP (Xiong et al., 2002). In mice treated with PB or TCPOBOP, the mRNA levels of CYP2B10, target gene of CAR, is about 2 to 7 times higher compared to that of control mice and the little change were observed in the baseline levels of bilirubin (data not shown). However, the injected bilirubin was rapidly eliminated after intravenous injection into the PB- or TCPOBOP-treated mice (Fig. 3). In particular, the serum level of bilirubin was recovered to its baseline level (0.2 mg/dL) by 10 min after the injection in mice treated with TCPOBOP. These results suggest that CAR regulates bilirubin-detoxifying enzymes and transporters. Next, we assessed the elimination of bilirubin in CIA mice. At 10 min, higher serum levels of total and indirect bilirubin were observed in CIA mice compared to control mice. Furthermore, the elimination of bilirubin was retarded for up to 60 min (Fig. 4). The serum level of direct bilirubin was significantly higher in CIA mice at 60 min than in control mice. The levels of serum bile acids and alanine aminotransferase did not differ among the groups treated with PB, TCPOBOP, DMSO and corn oil. The liver weight/body weight ratio was significantly increased in mice treated with TCPOBOP (data not shown).

It was unclear which enzymes and transporters varied in CIA mice, although CAR possibly participates in the regulation of bilirubin metabolism and excretion. It was also unknown whether all of the enzymes and transporters involved in the bilirubin clearance pathway were similarly impaired. To clarify these points, we determined the mRNA levels of the main factors involved in the bilirubin clearance pathway. As shown in Fig. 5, the mRNA levels of OATP2, GSTA1 and GSTA2 were dramatically decreased in CIA mice, while the mRNA levels of OATP4, UGT1A1 and Mrp2 remained unchanged. Moreover, the expression of OATP2 in the liver was determined by western blot analysis (Fig. 6). OATP2 expression levels in the liver of CIA mice were also reduced. In Fig. 7, the transport activities of OATP2 toward [³H]estrone 3-sulfate were shown. The significantly lower radioactivity was detected in hepatocytes derived from CIA mice at 5 min after initiation of uptake reaction at 37°C. On the other hand, the little change in radioactivity was observed in between control and CIA mice at 4°C. The little changes of total GSH levels were exhibited in livers of control and CIA mice [$750.9 \pm 31.8 \mu\text{M}$ and $781.6 \pm 55.3 \mu\text{M}$ in control and CIA mice, respectively (n = 4)]. The overall alterations in the related drug-metabolizing enzymes and transporters in CIA mice are summarized in Fig. 8.

Discussion

The results of the present study have indicated that alterations to nuclear receptors affect bilirubin detoxification during chronic arthritis in mice. Furthermore, we have demonstrated that decreased mRNA levels of CAR could inhibit the expressions of multiple enzymes and transporters involved in the bilirubin clearance pathway in chronic arthritis.

The CAR mRNA level was significantly decreased in the liver of CIA mice, whereas the mRNA levels of PXR and CCRP hardly changed. These results suggest that the expressions of enzymes and transporters are suppressed by decreased levels of CAR, rather than PXR, in CIA mice. The regulation mechanisms for the expressions of CAR and PXR are not yet fully understood. It is generally recognized that inflammatory cytokines, such as interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α , play critical roles in the negative regulation of CAR and PXR (Assenat et al., 2004; Fang et al., 2004; Pascussi et al., 2000). In CIA mice, the production of inflammatory cytokines is most likely to inhibit the expression of CAR although the precise reasons for the small alterations to PXR in CIA mice remain to be clarified. The signaling

pathway for the expression of CAR may be easily affected by chronic arthritis. We further suppose that the localization of CAR in hepatocytes could remain unchanged since little change in CCRP was observed in CIA mice.

We further investigated whether quantitative or functional changes in CAR affect bilirubin detoxication in chronic arthritis model mice. Bilirubin entry into the liver is mediated by OATP and bilirubin entered in liver is sequestered there by GSTA1 and A2, conjugated by UGT1A1, and then transported across the canalicular membrane by MRP2. In normal mice, the elimination of bilirubin was faster in mice treated with PB or TCPOBOP than in control mice, indicating that CAR induction could promote the expressions of enzymes and transporters involved in bilirubin clearance. In particular, the bilirubin level in TCPOBOP-treated mice was rapidly recovered to the baseline level by 10 min. This finding indicates that CAR is intimately involved in the regulation of bilirubin clearance, since TCPOBOP is known to be a strong inducer of CAR. Based on these results, we examined the bilirubin clearance in CIA mice, and found that the injected bilirubin persisted in the blood of CIA mice for a longer time compared to control mice. Accumulation of bilirubin in the body due to defects in bilirubin excretion results in hyperbilirubinemia and jaundice, and is commonly seen in genetic

Crigler-Najjar diseases and Dubin-Johnson syndrome (Jansen et al., 1995). Sustained hyperbilirubinemia may lead to cellular toxicity, including potentially lethal neurotoxicity. For these reasons, it is important to further investigate the changes in bilirubin clearance during chronic arthritis. Moreover, several previous reports have shown that the pharmacokinetics of propranolol, acebutolol and cyclosporine are altered during arthritis (Piquette-Miller et al., 1992; Piquette-Miller et al., 1995; Pollock et al., 1989; Walker et al., 1986).

Various factors are involved in the process of bilirubin uptake by hepatocytes toward biliary excretion. It was unclear which enzymes and drug transporters showed altered expressions in CIA mice, although the decreased CAR affected bilirubin clearance in these mice. To clarify this point, we examined the mRNA levels of some of the factors involved in the bilirubin clearance pathway, and found that the mRNA levels of OATP2, GSTA1 and GSTA2 were dramatically decreased in CIA mice. The protein levels of OATP2 were also decreased in CIA mice. Moreover, the uptake of estrone 3-sulfate into hepatocytes were decreased in CIA mice, indicating that the transport activities of OATP2 owing to the decrease in its protein levels could be decreased in CIA mice. GSTA1 and GSTA2 bind to bilirubin in hepatocytes and inhibit efflux of bilirubin into

the blood. Thus, the decreased mRNA levels of GSTA1 and GSTA2 may lead to higher efficiency of efflux from hepatocytes to the blood compared to the normal condition, although the expression of GSTA1 and A2 is not always relevance to their mRNA levels. The little alterations of total GSH levels exhibited between control and CIA mice although the mRNA levels of GSTA1 and GSTA2 were decreased in CIA mice. Consequently, as shown in Fig. 8 it is likely that the bilirubin clearance was suppressed, at least in part, due to the decreased activity of OATP2. Additionally, the reduction of GSTA1 and GSTA2 may contributes partially to the suppressed detoxification of bilirubin. In hepatocytes, the ingested bilirubin presumably underwent normal glucuronidation by UGT1A1 and biliary excretion by MRP2, since the expressions of these proteins hardly changed in CIA mice. The lack of change in the mRNA levels of OATP4, UGT1A1 and MRP2 in CIA mice suggests that these mRNA levels are regulated by other nuclear receptors, such as PXR. It is interesting to note that each enzyme and transporter has a distinct regulation pattern by CAR during chronic arthritis. From the results shown in Fig. 4, there are some possible explanations for the elimination of bilirubin in CIA mice. First, the levels of total and indirect bilirubin were higher at 10 min because the efficiency of the net uptake of bilirubin was decreased in CIA mice. In turn, the increased ratio of indirect to direct bilirubin

indicates that bilirubin delivery from the blood flow to hepatocytes was efficient, since the indirect bilirubin was not bound with protein in the liver. Second, the higher level of direct bilirubin at 60 min suggests that bilirubin could efflux to the blood flow in CIA mice. These results also indicate that the inhibition patterns by CAR could differ for each enzyme and drug transporter. CAR and PXR have been reported to have distinct target genes, although they regulate the overlapping of some target genes involved in all phases of xenobiotic metabolism (Maglich et al., 2002). Maglich et al. (2002) also identified the target genes of PXR and CAR in the liver and small intestine, namely GST by CAR, UGT1A1 by PXR and MRP2 by PXR and CAR. Our results are consistent with this report. The decreased levels of CAR inhibited the expression of GST during chronic arthritis. The mRNA levels of UGT1A1 and MRP2 exhibited little change in CIA mice probably due to being regulated by PXR. It is possible that the downregulation of CAR did not greatly affect the mRNA level of MRP2, since PXR and CAR both participate in the regulation of MRP2. It is also possible that the decreased level of OATP2 could affect the drug disposition of a substrate, such as pravastatine, in the liver during arthritis. A limitation of our study is that the changes in the nuclear receptors, enzymes and transporters at each phase of arthritis are unclear because we used the mice at 42 days after immunization. Thus, it is likely that PXR,

other enzymes and transporters were altered at the early stage of chronic arthritis. Furthermore, there is a possibility that the decreased levels of CAR inhibited other enzymes and transporters in CIA mice at each phase. In other inflammatory diseases, alterations to nuclear receptors may similarly affect the expressions of enzymes and transporters. Further studies are needed to clarify the alterations as well as the quantitative and functional changes to nuclear receptors under inflammatory conditions.

In conclusion, the mRNA expression of CAR target-genes OATP2 and GSTA1 and A2 as well as OATP2 activity were decreased in CIA mice while levels of OATP4, UGT1A1 and MRP2 remained unchanged. These alterations resulted in retarded elimination of bilirubin in CIA mice. This study provides important information regarding the effects of alterations in nuclear receptors on bilirubin-detoxifying enzymes and transporters in chronic arthritis. In particular, alterations in CAR in chronic arthritis affect the efficiency of bilirubin uptake by hepatocytes is decreased during chronic arthritis due to the downregulation of OATP2, GSTA1 and GSTA2.

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Footnotes

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Legends for figures

FIG. 1. Body weights and foot volumes of control and CIA mice up to 42 days after immunization. CIA mice were treated with bovine tracheal cartilage type II collagen emulsified in Freund's incomplete adjuvant in Bayol F oil under ether anesthesia. The hindpaw swelling of the left ankle was measured. The results are expressed as the mean \pm S.D. of 4 mice. * $p < 0.05$, significant difference between the values for CIA and control mice.

FIG. 2. mRNA levels of CAR, PXR and CCRP in the livers of control and CIA mice. The mRNA levels of CAR, PXR and CCRP were determined in the livers using an RT-PCR method. The results are expressed as the mean of each group following normalization by the corresponding level of 28S rRNA (n = 4). *** $p < 0.001$, significant difference between the values for CIA and control mice.

FIG. 3. Elimination of bilirubin after its intravenous injection into mice treated with PB or TCPOBOP. The concentrations of total, direct and indirect bilirubin were determined at 10 or 60 min after the injection. The results are expressed as the mean \pm

S.D. of 4 mice. *** $p < 0.001$, significant difference between the values for PB-treated or TCPOBOP-treated mice and control mice.

FIG. 4. Elimination of bilirubin after its intravenous injection into control and CIA mice. The concentrations of total, direct and indirect bilirubin were determined at 10 or 60 min after the injection. The results are expressed as the mean \pm S.D. of 4-6 mice. *** $p < 0.001$, significant difference between the values for control and CIA mice.

FIG. 5. mRNA levels of OATP4, OATP2, GSTA1, GSTA2, UGT1A1 and Mrp2 in the livers of control and CIA mice. The mRNA levels of OATP4, OATP2, GSTA1, GSTA2, UGT1A1 and Mrp2 were determined in the livers using an RT-PCR method. The results are expressed as the mean of each group following normalization by the corresponding level of 28S rRNA (n = 4). ** $p < 0.01$, significant difference between the values for CIA and control mice.

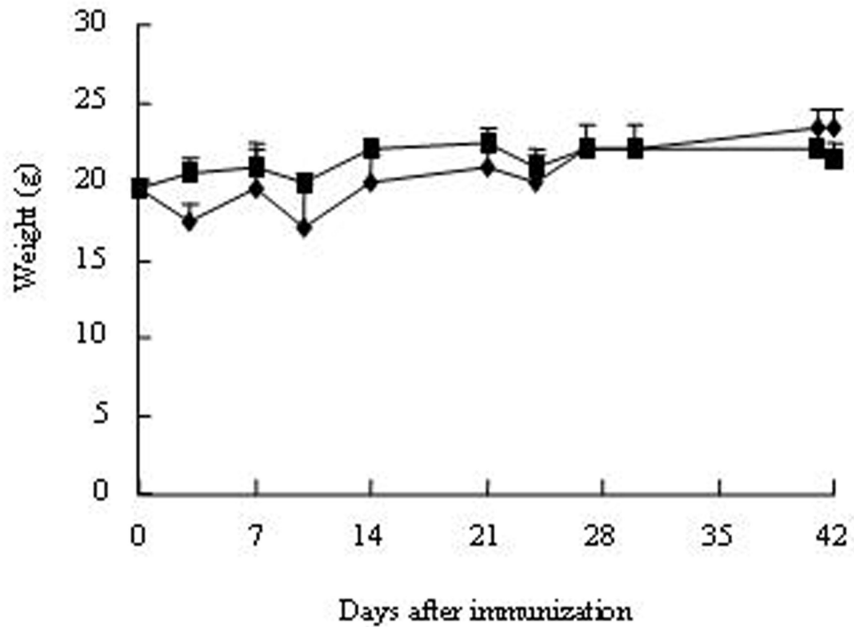
FIG. 6. Protein levels of OATP2 in the liver of control and CIA mice. Fifty micrograms of proteins was electrophoresed and then visualized. The arrow indicates OATP2 bands at 70 kDa.

FIG. 7. Transport study via OATP2 using mouse hepatocytes. The radioactivity of [³H]estrone 3-sulfate in hepatocytes were measured at 0.5, 2 and 5 min after initiation of reaction. The results are expressed as the mean \pm S.D. of 4-6 mice. ** $p < 0.005$, significant difference between the values for CIA and control mice.

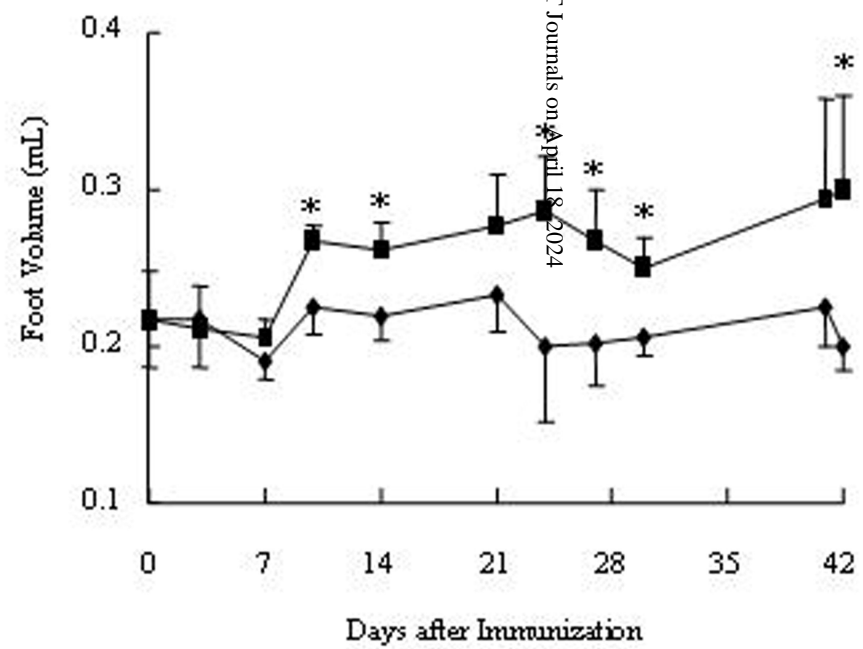
FIG. 8. Summary of the effects on the bilirubin clearance pathway in CIA mice. The mRNA levels of OATP2, GSTA1 and GSTA2 are decreased in CIA mice. B: bilirubin; B-GA: bilirubin glucuronide.

Fig. 1

Body Weight



Foot Volume



◆ Control
■ CIA

Fig. 2

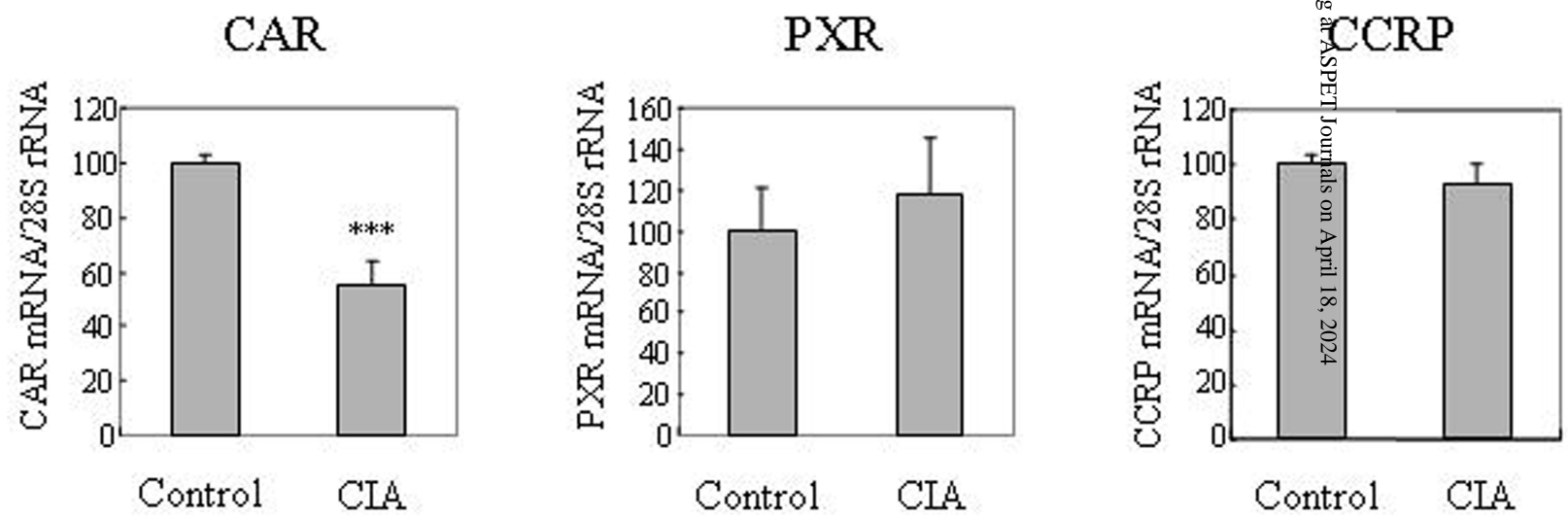


Fig. 3

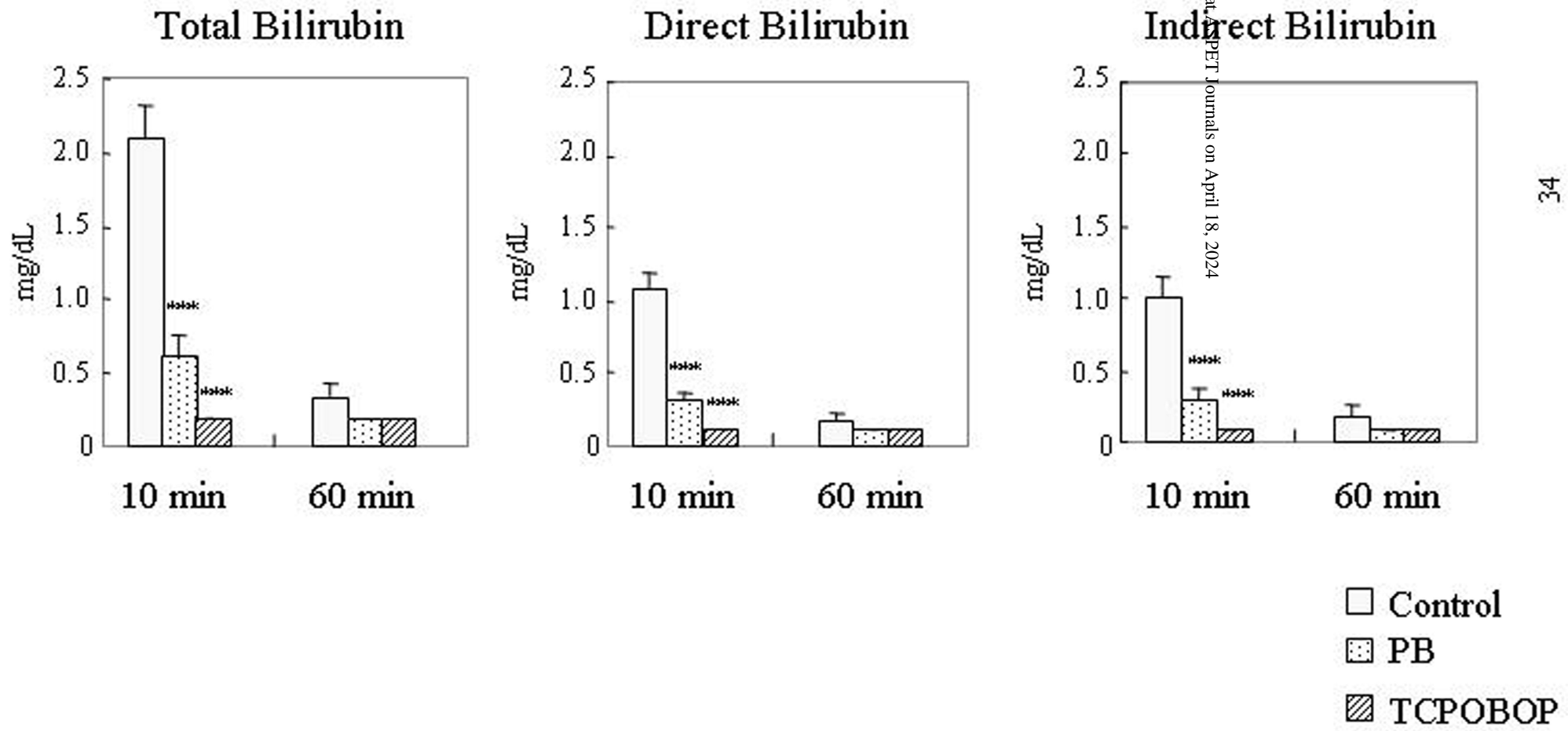


Fig. 4

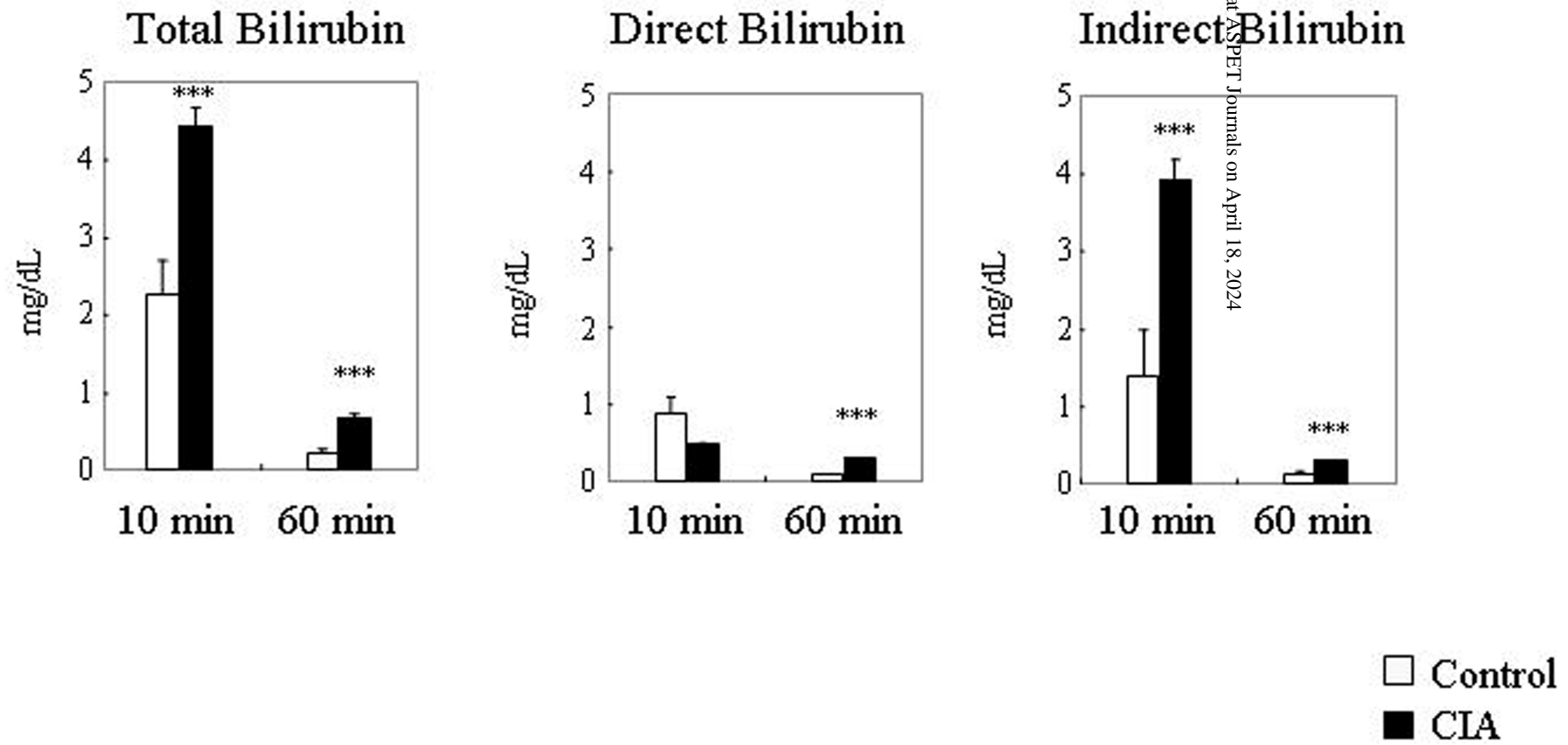


Fig. 5

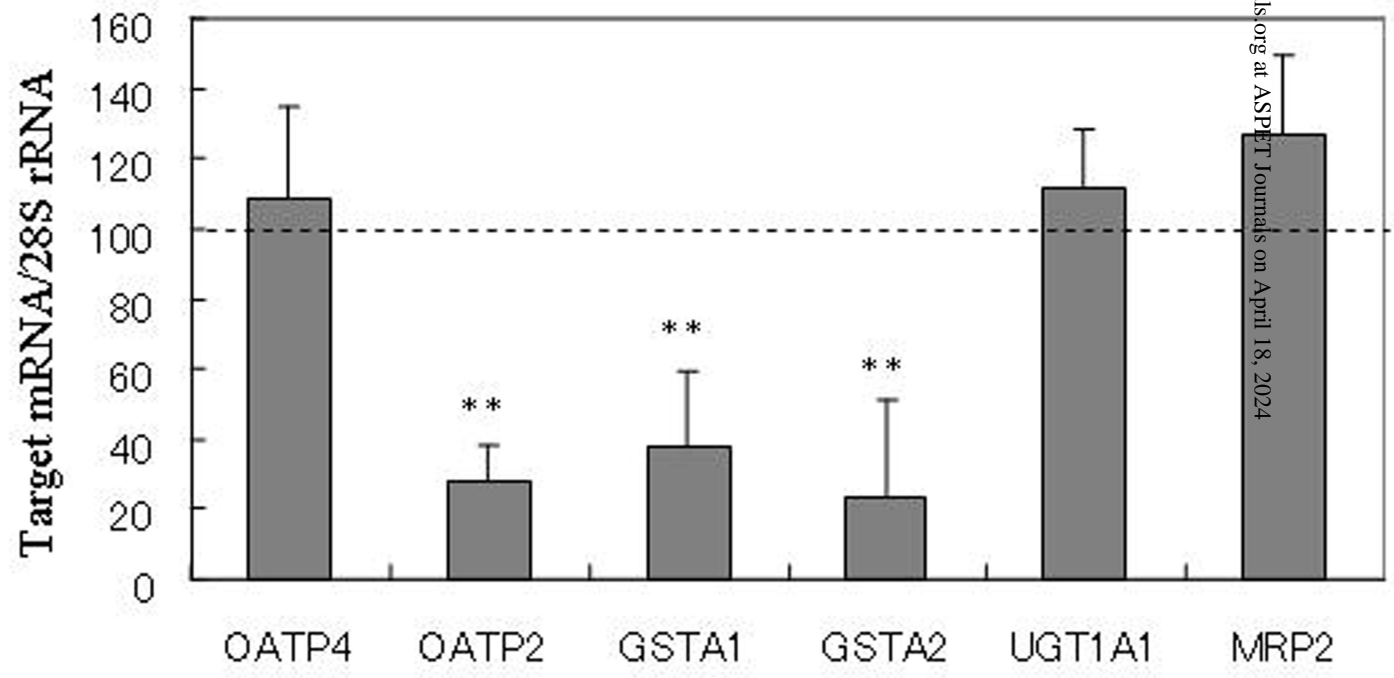


Fig. 6

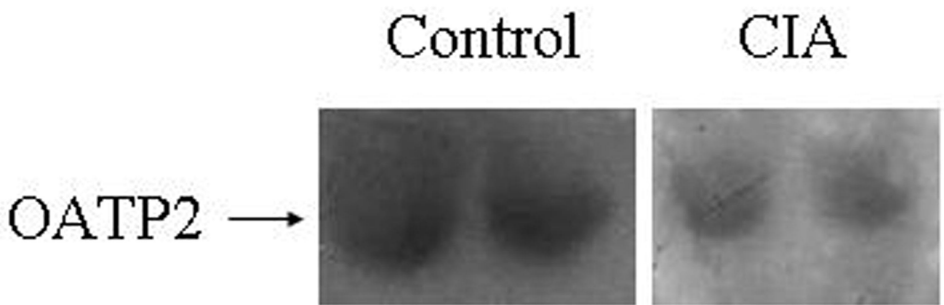
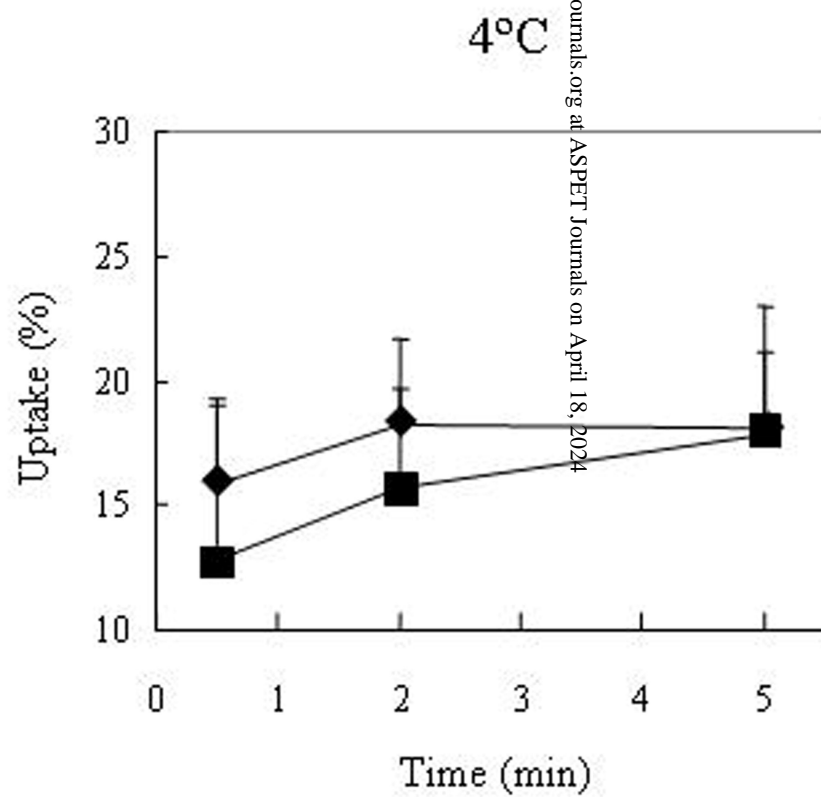
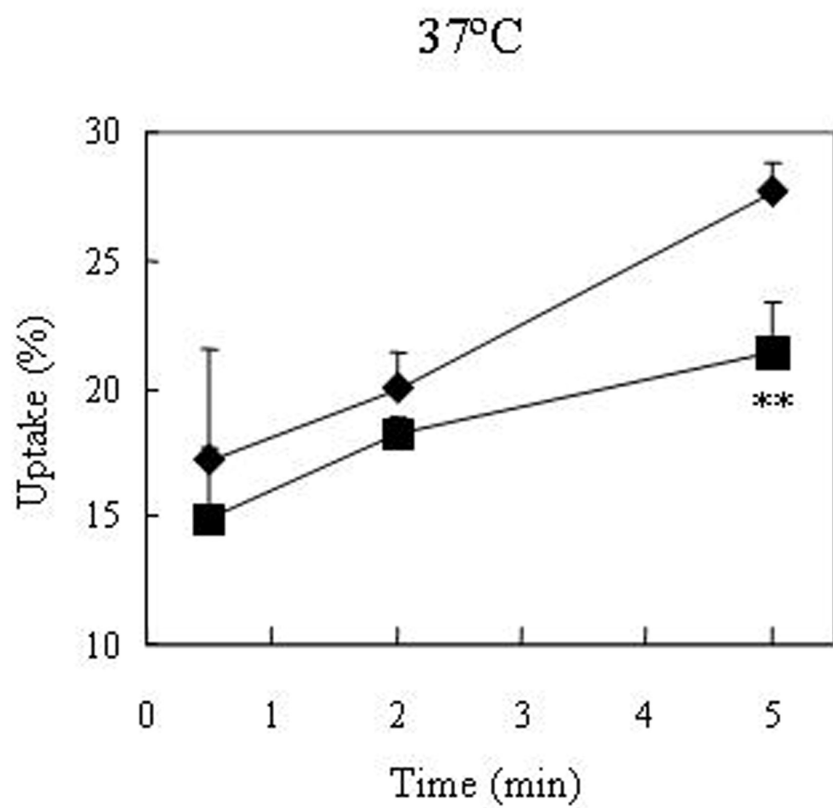


Fig. 7



◆ Control
■ CIA

Fig. 8

