Changes in the expression of drug-metabolising enzymes and drug transporters in mice with collagen antibody-induced arthritis

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ABSTRACT

1. We investigated the changes in the expression of drug-metabolising enzymes and drug transporters in the liver, small intestine and kidney of mice with collagen antibody-induced arthritis (CAIA) to determine whether changes in these expressions affect pharmacokinetics of drugs in patients with rheumatoid arthritis.

2. mRNA expression levels of cytochrome P450 (Cyp) 2b10, Cyp2c29 and Cyp3a11 were observed to be lower in the liver and small intestine of CAIA mice than in control mice. Compared with control mice, mRNA expression levels of multidrug resistance 1 b, peptide transporter 2 and organic anion transporter (Oat) 2 were high in the liver of CAIA mice. Changes in these expression levels were different among organs. However, elevated expression of Oat2 mRNA was not associated with an increase in protein expression and transport activity evaluated using [3H]cGMP as a substrate.

3. These results suggest that arthritis can change the expression of pharmacokinetics-related genes, but the changes may not necessarily be linked to the pharmacokinetics in patients with rheumatoid arthritis. On the other hand, we found Oat2 mRNA expression level was positively correlated with plasma interleukin-6 level, indicating that transcriptional activation of Oat2 may occur in inflammatory state.

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Introduction

Inflammation affects the pharmacokinetics of various drugs, and previous studies have investigated the changes in the expression of drug-metabolising enzymes and drug transporters in the inflammatory state in vivo and in vitro (Coutant and Hall 2018; Wu and Lin 2019; Stanke-Labesque et al. 2020; Saib and Delavenne 2021). These include cytochrome P450 (CYP) 1A2, CYP2C19, CYP3A4, multidrug resistance (MDR) 1, multidrug resistance-associated protein (MRP) 2 and breast cancer resistance protein (BCRP), which are downregulated in the inflammatory state (Coutant and Hall 2018; Wu and Lin 2019; Stanke-Labesque et al. 2020; Saib and Delavenne 2021), mainly attributed to inflammatory cytokines such as interleukin (IL) and tumour necrosis factor (TNF). Treatment with IL-1β, IL-6, or TNF-α decreased the mRNA and protein expression levels of various drug-metabolising enzymes and drug transporters in human primary hepatocytes (Aitken and Morgan 2007; Le Vee et al. 2009), mouse hepatocytes (Dickmann et al. 2012) and rat hepatocytes (Sukhai et al. 2001; Lee et al. 2009).

Rheumatoid arthritis (RA) is an inflammatory disease that can affect the pharmacokinetics of drugs in patients. The area under the concentration-time curve (AUC) of simvastatin and verapamil, which are CYP3A substrates, in patients with RA was significantly higher than that in healthy subjects (Coutant and Hall 2018). On the other hand, several reports have suggested that pharmacokinetics of certain drugs that are substrates of drug-metabolising enzymes and drug transporters were not different in patients with RA (Coutant and Hall 2018; Ono et al. 2021). Additionally, the relationship between changes in the expression of these proteins and their function in patients with RA remains unclear. It is important to clarify the effects of changes in the expression of these proteins on the pharmacokinetics. A collagen antibody-induced arthritis (CAIA) mouse was developed as an animal model of RA. The CAIA model has several advantages compared to the classical model of collagen-induced arthritis (CIA) such as high incidence rate, rapid disease onset, and the ability to use a wide range of strains and genetically modified mice (Khachigian 2006). It has been reported that various CYP isoforms, such as Cyp1a2, Cyp2b10, Cyp2c29, Cyp3a11 were downregulated and the enzymatic activity of the corresponding CYP was decreased in the liver of mice with CAIA (Dickmann et al. 2012). However, changes in the expression levels of drug-metabolising enzymes and drug transporters in the liver and extrahepatic organs have not been comprehensively elucidated; it needs to be clarified because RA is characterised by systemic inflammation, and drug-metabolising enzymes and drug transporters that...
contribute to the pharmacokinetics of drugs are expressed in the small intestine and kidney as well as in the liver.

In this study, we examined the variations in gene expression of drug transporters and drug-metabolising enzymes in the liver, small intestine and kidney of mice with CAIA. We found that arthritis affects the expression of drug-metabolising enzymes and drug transporters, depending on the organ. These findings may be useful in considering the systemic pharmacokinetics of drugs in patients with RA.

Materials and methods

Animals

Seven-week-old female BALB/c mice were obtained from Japan SLC, Inc. (Shizuoka, Japan). Mice were housed in Specific Pathogen Free condition under a 12-h light/dark cycle and with free access to tap diet and water. All mouse experiments were approved by the Animal Research Committee of Ritsumeikan University (approval date: 4 February 2020, document number: BKC2019-039).

Collagen antibody-induced arthritis (CAIA) model

CAIA model was produced according to the manufacturer’s instructions. Mice (7–8 weeks old) were randomly assigned to each group and intraperitoneally injected with 1.5 mg/0.15 mL of collagen antibody cocktail (CAIA group; Chondrex, Redmond, WA, USA) or 0.15 mL of phosphate-buffered saline (PBS) as a vehicle [lipopolysaccharide (LPS) group and control group] on day 0. On day 3, the mice were intraperitoneally injected with 25 μg/0.05 mL of LPS (CAIA and LPS groups) or 0.05 mL of PBS (control group). The LPS group was created as a comparison group according to the manufacturer’s instructions. On day 7, the mice were anaesthetised under isoflurane, and the blood (with heparin), liver, small intestinal epithelium (jejunum) and kidney (without capsule) were collected. Body weight and arthritis score were measured once a day. The arthritis score was assigned based on visual evaluation according to the manufacturer’s instructions (Chondrex) as follows: score 0, normal; score 1, any one joint among interphalangeal joint, metacarpophalangeal joint and ankle; score 2, both interphalangeal and metacarpophalangeal joints; score 3, both interphalangeal and ankle; and score 4, the entire paw is red, swollen, and hot. The scores for each paw were summed, and the maximum score was 16.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The mRNA levels of each drug transporter and drug-metabolising enzyme were determined by RT-qPCR. RNA later Solution (Thermo Fisher Scientific, Waltham, MA, USA) was used when the liver, small intestine and kidney tissues were collected, and TRIzol reagent (Thermo Fisher Scientific) was used to isolate total RNA. Reverse transcription was conducted using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) with 2 μg of total RNA. Quantitative PCR was performed using StepOne Real-Time PCR System (Thermo Fisher Scientific) using a mixture of PowerUp SYBR Green Master Mix (Thermo Fisher Scientific), cDNA and each primer. The thermocycling conditions were as follows: 50 °C for 2 min, 95 °C for 2 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min, 60–95 °C at a rate of 0.3 °C/s for melting curve acquisition. The primers used are listed in Supplementary Table S1. The specificity of all primers was confirmed by in silico and in vitro agarose gel electrophoresis. No amplification with genomic DNA and 100 ± 20% amplification efficiency based on the slope of the standard curve were also confirmed. The ΔΔCT method was employed to calculate the relative expression levels of target genes. As the reference gene, glyceraldehyde 3-phosphate dehydrogenase (Gapdh) was used.

Organic anion transporter (OAT) 2-mediated uptake assay

The transport activity of mouse Oat2 was evaluated using primary mouse hepatocytes, which were isolated using a two-step collagenase liver perfusion method (Klaunig et al. 1981). Hepatocytes were seeded at a density of 5 × 10⁵ cells/ml/well in 12-well plates coated with collagen (Type I, Nitta Gelatine Inc., Osaka, Japan). Cells were cultured in DMEM/F12 containing 10% foetal bovine serum, 10 mM nicotinamide, 2 mM L-glutamine, 100 nM dexamethasone, 50 μM 2-mercaptoethanol, 1 μg/mL insulin, 520 μM L-ascorbic acid, 100 units/mL penicillin, 100 μg/mL streptomycin and 10 mM HEPES. Hepatocytes were used for the uptake assay after 4–6 h of seeding. The culture medium was removed and replaced with an incubation medium (3 mM KCl, 145 mM NaCl, 0.5 mM MgCl₂–6H₂O, 1 mM CaCl₂–2H₂O, 5 mM d-Glucose and 5 mM HEPES). After pre-incubation for 10 min at 37 °C, the medium was replaced with an incubation medium containing [³H]cGMP (PerkinElmer, Inc., Boston, MA, USA). The incubation condition of uptake assay was 100 nM [³H]cGMP as substrate concentration in time dependence experiment and for 1 min as incubation time in concentration dependence experiment. After incubation, the cells were washed twice with ice-cold incubation medium and 0.5 M NaOH was added to lyse the cells. The lysates were neutralised, and radioactivity was detected using a liquid scintillation counter (Beckman Coulter, Inc., Brea, CA, USA). The protein content of the solubilised cells was determined using Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and the uptake (mol) was normalised to the protein content.
**SDS-PAGE and Western blotting**

The protein expression of Oat2 was examined by Western blotting. The liver was homogenised using 1.15% KCl for the whole liver homogenates or 250 mM sucrose in 50 mM Tris-HCl buffer (pH 7.4) for the crude membrane fraction. The homogenates for the crude membrane fraction were centrifuged for 10 min at 3,000 g, and the supernatants were centrifuged for 30 min at 15,000 g. The pellets were suspended in 50 mM mannitol in 50 mM Tris-HCl buffer (pH 7.4). The protein content was then determined using Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Inc.). The liver samples (60 µg of protein per lane) were electrophoretically separated on 10% polyacrylamide gel at 200 V. Then, the proteins were transferred to PVDF membranes (Merck KGaA, Darmstadt, Germany) for 45 min at 15 V and blocked with 5% skim milk in TBS-T [0.15 M NaCl, 0.1% Tween 20 and 10 mM Tris-HCl (pH 8.0)] for 1 h at room temperature. After blocking, the membranes were incubated with the following primary antibodies at 4 °C: SLC22A7 rabbit polyclonal antibody (1/1,000, Proteintech Group, Inc., Rosemont, IL, US), ATP1A1 (Na+/K+-ATPase) rabbit polyclonal antibody (1/10,000, Proteintech Group, Inc.) and anti-GAPDH monoclonal antibody (1/2,000, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The membranes were washed with TBS-T and incubated with goat anti-rabbit IgG (whole molecule)-peroxidase-conjugated AffiniPure goat anti-mouse IgG (H + L) (1/10,000, Proteintech Group, Inc.) for 1 h at room temperature. The bound antibody was detected and quantified using ImmunoStar™ LD (FUJIFILM Wako Pure Chemical Corporation) and Amersham Imager 680 (GE Healthcare Japan, Tokyo, Japan). Protein expression was normalised to that of GAPDH (whole liver) or ATP1A1 (Na+/K+-ATPase, crude membrane fraction).

**Statistical analysis**

All data were presented as the mean ± S.D. Statistical analysis was carried out using GraphPad Prism version 8.4.3 (GraphPad Software, La Jolla, CA, USA) using one-way ANOVA with Tukey’s multiple comparisons test or Pearson correlation analysis. Statistical analysis was also carried out in Microsoft Excel using Student’s t-test. Statistical significance was set at P < 0.05.

**Results**

**Body weight, arthritis score and plasma cytokine level in mice with CAIA**

We used anti-collagen antibodies and LPS to mice to establish a mouse model of CAIA. We administered PBS twice or PBS and LPS to mice of the control group or comparison group (LPS group), respectively. The mean value of body weight in each group was maintained after the first administration but decreased after LPS administration (Figure 1(A)). Arthritis score is an index of severity of arthritis. In the CAIA group, arthritis was observed from day 3, and arthritis score gradually increased from day 3 to day 7. On day 7, arthritis score was 6.4 ± 2.1 compared to the maximum score of 16 (Figure 1(B)). Arthritis was not observed in the control and LPS groups during the experimental period (Figure 1(B)). Figure 1(C) shows the plasma levels of IL-1β, IL-6 and TNF-α in each group on day 7. Higher IL-1β level was observed in the CAIA group as compared with that in the LPS group, although the level was low in the two CAIA mice. IL-6 level was well identified in the CAIA group and was significantly higher than that in the control and LPS groups. TNF-α level did not differ among the three groups (Figure 1(C)).

**mRNA expression of CYP isoforms and nuclear receptors in the liver, small intestine and kidney of mice with CAIA**

Then, the effect of CAIA on the mRNA expression levels of CYP isoforms and nuclear receptors, which are transcription factors that regulate CYP isoforms and drug transporters, were investigated in the liver, small intestine and kidney. In the liver, the mRNA expression levels of Cyp1a2, Cyp2b10, Cyp2c29 and Cyp3a11 were significantly lower in the CAIA group than in the control group. Decreased expression of Cyp3a11 was observed in the CAIA group than in the LPS group. Cyp3a13 expression level was significantly higher in the CAIA group than in the control and LPS groups. Pregnane X receptor (Pxr) expression levels were higher in the CAIA group than in the LPS group (Figure 2(A)). In the small intestine, Cyp2b10, Cyp2c29 and Cyp3a11 mRNA expression tended to be lower than in the CAIA group than in the control group, similar to that in the liver (Figure 2(B)). In the kidney that do not express several CYP isoforms, Cyp2d22 and Cyp2e1 expression levels in the CAIA group did not differ from those in the control group, similar to those in the small intestine and liver. Moreover, constitutive androstane receptor (Car) expression was significantly lower in the CAIA group than in the control group (Figure 2(C)).

**mRNA expression of drug transporters in the liver, small intestine and kidney of mice with CAIA**

Figure 3 shows the mRNA expression levels of each drug transporter in the liver, small intestine and kidney of mice with CAIA. In the liver, higher mRNA expression of Mdr1b, peptide transporter (Pept) 2 and Oat2 was observed in the CAIA group than in the control group. The expression levels of Mrp2, Mdr1a, bile salt export pump (Bsep), Bcrp, organic anion transporting polypeptide (Oatp) 1b2, Oatp2b1, organic cation transporter (Oct) 1 and multidrug and toxin extrusion (Mate) 1 did not differ among the three groups (Figure 3(A)). In the small intestine, decreased mRNA expression of Bcrp was observed in the CAIA group as compared with in the control and LPS groups (Figure 3(B)). Mdr1b mRNA expression was significantly lower in the small intestine and kidney of CAIA mice than in the control group, contrary to that in the liver (Figure 3(B,C)).

The hepatic mRNA expression levels of Mdr1b, Pept2 and Oat2 were high in the CAIA group (Figure 3(A)). The
intestinal and renal mRNA expression levels of Mdr1b and
the intestinal expression levels of Bcrp were low in the CAIA
group (Figure 3(B,C)). Then, the mRNA expression levels and
plasma IL-6 levels in the individual mice of control, LPS and
CAIA groups were plotted as correlation diagram to evaluate
the relationship between the mRNA expression levels of
these drug transporters and the degree of inflammation. The
hepatic mRNA expression level of Oat2 was positively corre-
lated with plasma IL-6 level (Figure 4). On the other hand,
the hepatic mRNA expression levels of Mdr1b and Pept2
were not correlated with plasma IL-6 level (Supplementary
Figure S1). The intestinal mRNA expression level of Mdr1b and Bcrp was negatively correlated with plasma IL-6 level (Supplementary Figure S1). The renal mRNA expression level of Mdr1b was not correlated with plasma IL-6 level (Supplementary Figure S1).

**OAT2-mediated transport of cGMP in hepatocytes of mice with CAIA**

The mRNA expression level of Oat2, an uptake transporter,
increased significantly in the liver of mice with CAIA (Figure
3(A)) and the individual mRNA expression level was
correlated with plasma IL-6 level (Figure 4). Therefore, we
evaluated the transport activity of OAT2 in mouse primary
hepatocytes using [3H]cGMP as a substrate. Figure 5(A)
shows the time dependence of cGMP uptake in mouse pri-
mary hepatocytes. cGMP transport activity did not differ
between the hepatocytes of CAIA mice and control mice.
Next, we examined the concentration dependence of cGMP
uptake (Figure 5(B)). However, saturation was not observed
in the concentration-dependent experiment.

**Protein expression of OAT2 in the whole liver and crude
membrane fraction of mice with CAIA**

Despite the high expression levels of Oat2 mRNA in the liver
of CAIA mice, transport activity of OAT2 did not change
(Figures 3(A) and 5). Therefore, the protein expression of
OAT2 in the whole liver and crude membrane fraction of
CAIA mice was examined. In the whole liver, the protein
expression of OAT2 in the CAIA group was similar to that in
the control and LPS groups. However, as compared to the
control group, the protein expression of OAT2 tended to
decrease in the CAIA and LPS groups in the crude membrane
fraction of the liver (Figure 6).
Discussion

We investigated the changes in the expression of drug-metabolising enzymes and drug transporters in the liver, small intestine and kidney of CAIA mice to determine whether changes in these expressions affect pharmacokinetics of drugs in patients with rheumatoid arthritis. First, we confirmed the severity of arthritis and degree of inflammation in CAIA mice (Figure 1). Arthritis score and plasma cytokine level in CAIA mice were similar to those in a previous study (Dickmann et al. 2012). The IL-6 levels were not different from those in patients with RA (Kobayashi et al. 2010; Chung et al. 2011; Adlan et al. 2015). TNF-α level was low near the limit of detection. Under these conditions, the mRNA expression of Cyp1a2, Cyp2b10, Cyp2c29, Cyp3a11, Cyp3a13 and Pxr was changed in the liver of mice with CAIA, consistent with previous report (Figure 2; (Dickmann et al. 2012). The mRNA expression levels of CYP isoforms and nuclear receptors were evaluated by RT-qPCR. The relative expression levels were normalised to the expression level of Gapdh as the reference gene and calculated relative to those in the control group. Each column represents the mean ± S.D. (n = 5); *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group; †p < 0.05, ††p < 0.01, †††p < 0.001 vs. LPS group (Tukey’s multiple comparison test). AhR: aryl hydrocarbon receptor; Car: constitutive androstane receptor; Cyp: cytochrome P450; Pxr: pregnane X receptor.

Figure 2. mRNA expression levels of CYP isoforms and nuclear receptors in the liver (A), small intestine (B) and kidney (C) of control, LPS and CAIA mice. The mRNA expression levels of CYP isoforms and nuclear receptors were evaluated by RT-qPCR. The relative expression levels were normalised to the expression level of Gapdh as the reference gene and calculated relative to those in the control group. Each column represents the mean ± S.D. (n = 5); *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group; †p < 0.05, ††p < 0.01, †††p < 0.001 vs. LPS group (Tukey’s multiple comparison test). AhR: aryl hydrocarbon receptor; Car: constitutive androstane receptor; Cyp: cytochrome P450; Pxr: pregnane X receptor.
et al. 2012). We also showed the altered mRNA expression of CYP isoforms in the small intestine and kidney of CAIA mice. In rats with CIA, a classical animal model of RA, mRNA expression of Cyp3a1 was decreased in the small intestine (Lin et al. 2017). Thus, arthritis affects the expression levels of CYP in the small intestine, kidney and liver. In addition, changes in the mRNA expression levels of several drug transporters were observed in mice with CAIA and were different among the organs examined (Figure 3). In rats with adjuvant-induced arthritis, another classical animal model for RA, the changes in the expression of several ABC transporters, Mdr1a, Mdr1b, MRP2 and Bcrp were different among organs such as the liver, small intestine, kidney and brain (Kawase et al. 2014). Effects of arthritis on the expression of drug transporters are different among organs, although the mechanism remains unclear. It is possible that systemic

Figure 3. mRNA expression levels of drug transporters in the liver (A), small intestine (B) and kidney (C) of control, LPS and CAIA mice. The mRNA expression levels of drug transporters were evaluated by RT-qPCR. The relative expression levels of drug transporters were normalised to the expression level of Gapdh as the reference gene and calculated relative to those in the control group. Each column represents the mean ± S.D. (n = 5); *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group; ††p < 0.01 vs. LPS group (Tukey’s multiple comparison test). BCRP: breast cancer resistance protein; BSEP: bile salt export pump; MATE: multidrug and toxin extrusion; MDR: multidrug resistance; MRP: multidrug resistance-associated protein; OAT: organic anion transporter; OATP: organic anion transporting polypeptide; OCT: organic cation transporter; PEPT, peptide transporter.
inflammation causes different effects on each organ, and the regulation of drug transporter expression differs in each organ.

The changes in the expression of drug-metabolising enzymes and drug transporters due to inflammation are mainly attributed to inflammatory cytokines such as IL-1β, IL-6 and TNF-α. In human and mouse primary hepatocytes, these cytokines showed potential to decrease the expression levels of various drug transporters and drug-metabolising enzymes (Aitken and Morgan 2007; Le Vee et al. 2009; Dickmann et al. 2012). However, unlike previous in vitro studies (Hisaeda et al. 2004; Le Vee et al. 2009; Diao et al. 2010), downregulation of Mrp2, Bsep, Oatp, Oat and Oct was not observed in our results. The inflammatory response or regulation of each gene expression may differ between in vitro and in vivo studies. Inflammation-induced downregulation of drug transporters and drug-metabolising enzymes has been reported to be caused by the activation of nuclear factor (NF)-κB, a transcription factor that responds to inflammatory cytokines (Wu and Lin 2019). The downregulation observed in our results might be caused by NF-κB activation. However, the mechanism underlying the upregulation of Mrp1b, Pept2 and Oat2 in the liver of CAIA mice is not clear. Further studies are needed to clarify the mechanism underlying the changes in the expression of drug transporters and drug-metabolising enzymes in the inflammatory state in vivo.

OAT2 is mainly expressed in the liver and kidney, and is a solute carrier transporter that contributes to the uptake of several drugs, including ibuprofen, diclofenac, warfarin and tolbutamide, in the human liver (Bi, Lin, et al. 2018; Bi, Mathialagan, et al. 2018; Kimoto et al. 2018). In our results, the mRNA expression level of Oat2 was significantly higher in the liver of CAIA mice; however, the protein expression and transport activity of OAT2 were not correlated (Figures 3(A), 5 and 6). It has been reported that the protein expression and transport activity of several drug transporters do not necessarily correlate with the corresponding mRNA expression for several reasons, including post-transcriptional modification, intracellular trafficking and membrane localisation (Ohtsuki et al. 2012; Li et al. 2019). From our results in Figure 6, mouse OAT2 localisation is not clear at this stage. The mechanism is not clear, and further investigation is needed to clarify post-transcriptional modification, intracellular trafficking and membrane localisation of OAT2 in CAIA mice. We then examined the transport activity of OAT2 using cGMP as a probe substrate (Cropp et al. 2008; Marada et al. 2015). Saturation of transport activity was not observed in concentration dependence experiment of cGMP uptake (Figure 5(B)), although the uptake of 100 nM [3H]cGMP was decreased by 38.8 ± 13.2% by an OAT2 inhibitor, indomethacin, at 100 μM in our preliminary experiment using normal mice (data not shown). The basal transport activity of OAT2 may be low in mouse hepatocytes. It was reported that Oat2 regulation of each gene expression may differ between in vitro and in vivo studies. Inflammation-induced downregulation of drug transporters and drug-metabolising enzymes has been reported to be caused by the activation of nuclear factor (NF)-κB, a transcription factor that responds to inflammatory cytokines (Wu and Lin 2019). The downregulation observed in our results might be caused by NF-κB activation. However, the mechanism underlying the upregulation of Mrp1b, Pept2 and Oat2 in the liver of CAIA mice is not clear. Further studies are needed to clarify the mechanism underlying the changes in the expression of drug transporters and drug-metabolising enzymes in the inflammatory state in vivo.

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**Figure 4.** Correlation between hepatic Oat2 mRNA expression levels and plasma IL-6 levels in the individual mice of control, LPS and CAIA groups. The hepatic mRNA expression levels of Oat2 (vertical line) and plasma IL-6 levels (horizontal line) was plotted. The individual data in Figures 1 and 2 were used. Pearson correlation analysis was performed.

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**Figure 5.** Time dependence (A) and concentration dependence (B) of cGMP uptake in the primary hepatocytes of control and CAIA mice. The transport activity of Oat2 in mouse primary hepatocytes was evaluated using [3H]cGMP as a specific substrate; substrate concentration was 100 nM [3H]cGMP in the time dependence experiment, and incubation time was 1 min in the concentration dependence experiment. The transport activity was normalised to the protein content of the hepatocytes. Each point represents the mean ± S.D. (n = 3); *p < 0.05 vs. control group (Student’s t-test).
mRNA expression was significantly increased in mice fed a high-fat diet (He et al. 2020) and in pigs with cadmium-induced liver injury (Wang et al. 2021), although the protein expression and transport activity were not determined. In addition, decreased expression level of OAT2 indicated a high risk of hepatocellular carcinoma in patients with chronic hepatitis C (Yasui et al. 2014). It has been speculated that Oat2 is related to embryonic development of various tissues in mice (Pavlova et al. 2000). Therefore, OAT2 may play important pathological and physiological roles, regardless of its transport function. From our results of positive correlation between the individual mRNA expression level of Oat2 and plasma IL-6 level (Figure 4), Oat2 gene expression may be regulated by inflammation. Further investigation of the role of OAT2 under pathological and physiological conditions is needed.

Our results of the changes in the pharmacokinetics-related genes can associate with their function, and affect pharmacokinetics of certain drugs in patients with RA. In patients with RA, the pharmacokinetics of CYP3A substrates, such as simvastatin and verapamil, have been characterised. The AUC of simvastatin was approximately 3.5-fold higher than that in healthy subjects (Coutant and Hall 2018), whereas the AUC of simvastatin was decreased by infusion of tocilizumab, an anti-human IL-6 receptor antibody, suggesting that IL-6 was responsible to reduce CYP3A activity in patients with RA (Schmitt et al. 2011). The AUC of verapamil in patients with RA was 3–4-fold higher than that in healthy subjects (Mayo et al. 2000). Serum levels of IL-1ra, IL-6 and IL-8 in patients with RA are negatively correlated with the serum concentration of 4β-hydroxycholesterol, which is an endogenous metabolite formed by CYP3A4/5 (Wollmann et al. 2018). Our results on the mRNA expression of Cyp3a11 (comparable to human CYP3A4) in the liver of CAIA mice supported these findings. However, the clinical significance of the changes in the expression of drug transporters or drug-metabolising enzymes except CYP3A has not been fully clarified in patients with RA. Whether change in the expression of drug transporters and drug-metabolising enzymes directly leads to a change in their function should be examined. RA may affect the pharmacokinetics of multiple substrates of drug transporters and drug-metabolizing enzymes because their gene expression profiles were altered in our results of CAIA mice.

In conclusion, we demonstrated the changes in the gene expression of drug transporters and drug-metabolising enzymes in the liver, small intestine and kidney of CAIA mice. Gene expression levels differed significantly between CAIA mice and control mice. The changes in gene expression of CYP isosforms in the CAIA group were similar among organs, whereas the changes in gene expression of several drug transporters in the CAIA group were different among organs. The significant increase in gene expression of Oat2 in the liver of CAIA mice was not linked to protein expression and transport activity. The expression of several drug transporters and drug-metabolizing enzymes found in this study may variously affect the pharmacokinetics in patients with RA.

Disclosure statement
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References
Adlan AM, Panoulas VF, Smith JP, Fisher JP, Kitas GD. 2015. Association between corrected QT interval and inflammatory cytokines in rheumatoid arthritis. J Rheumatol. 42(3):421–428.
Alitken AE, Morgan ET. 2007. Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metab Dispos. 35(9):1687–1693.
Bi Y-A, Lin J, Mathialagan S, Tylaska L, Callegari E, Rodrigues AD, Varma MVS. 2018. Role of hepatic organic anion transporter 2 in the pharmacokinetics of R- and S-warfarin: in vitro studies and mechanistic evaluation. Mol Pharm. 15(3):1284–1295.
Bi Y-A, Mathialagan S, Tylaska L, Fu M, Keefe J, Vldhede A, Costales C, Rodrigues AD, Varma MVS. 2018. Organic anion transporter 2 mediates hepatic uptake of tolbutamide, a CYP2C9 probe drug. J Pharmacol Exp Ther. 364(3):390–398.
Chung S-J, Kwon Y-J, Park M-C, Park Y-B, Lee S-K. 2011. The correlation between increased serum concentrations of interleukin-6 family cytokines and disease activity in rheumatoid arthritis patients. Yonsei Med J. 52(1):113–120.
Coutant DE, Hall SD. 2018. Disease-drug interactions in inflammatory states via effects on CYP-mediated drug clearance. J Clin Pharmacol. 58(7):849–863.

Cropp CD, Komori T, Shima JE, Urban TJ, Yee SW, More SS, Giacomini KM. 2008. Organic anion transporter 2 (SLC22A7) is a facilitative transporter of cGMP. Mol Pharmacol. 73(4):1151–1158.

Diao L, Li N, Brayman TG, Hotz KJ, Lai Y. 2010. Regulation of MRPs/ABCC2 and BSEP/ABCB11 expression in sandwich cultured human and rat hepatocytes exposed to inflammatory cytokines TNF-α [alpha], IL-6, and IL-1β [beta]. J Biol Chem. 285(41):31185–31192.

Dickmann LJ, McBride HJ, Patel SK, Miner K, Winkers LC, Slatter JG. 2012. Murine collagen antibody induced arthritis (CAIA) and primary mouse hepatocyte culture as models to study cytochrome P450 suppression. Biochem Pharmacol. 83(12):1682–1689.

He Y, Yang T, Du Y, Qin L, Ma F, Wu Z, Ling H, Yang L, Wang Z, Zhou Q, et al. 2020. High fat diet significantly changed the global gene expression profile involved in hepatic drug metabolism and pharmacokinetic system in mice. Nutr Metab (Lond). 17:37.

Hisaeda K, Inokuchi A, Nakamura T, Iwamoto Y, Kohno K, Kuwano M, Uchiumi T. 2004. Interleukin-1β represses MRPs gene expression through inactivation of interferon regulatory factor 3 in HepG2 cells. Hepatology. 39(6):1574–1582.

Kawase A, Norikane S, Okada A, Adachi M, Kato Y, Iwaki M. 2014. Distinct alterations in ATP-binding cassette transporter expression in liver, kidney, small intestine, and brain in adjuvant-induced arthritic rats. J Pharm Sci. 103(8):2556–2564.

Khachigian LM. 2006. Collagen antibody-induced arthritis. Nat Protoc. 1(5):2512–2516.

Kimoto E, Mathialagan S, Tylaska L, Niosi M, Lin J, Carlo AA, Tess DA, Varma MVS. 2018. Organic anion transporter 2-mediated hepatic uptake contributes to the clearance of high-permeability-low-molecular-weight acid and zwitterion drugs: evaluation using 25 drugs. J Pharmacol Exp Ther. 367(2):322–334.

Klaunig JE, Goldblatt PJ, Hinton DE, Lipsky MM, Chacko J, Trump BF. 1981. Mouse liver cell culture. I. Hepatocyte isolation. In Vitro. 17(10):913–925.

Kobayashi T, Murasawa A, Komatsu Y, Yokoyama T, Ishida K, Abe A, Yamamoto K, Yoshie H. 2010. Serum cytokine and periodontal profiles in relation to disease activity of rheumatoid arthritis in Japanese adults. J Periodontol. 81(5):650–657.

Le Vee M, Leecureur V, Stieger B, Fardel O. 2009. Regulation of drug transporter expression in human hepatocytes exposed to the proinflammatory cytokines tumor necrosis factor-alpha or interleukin-6. Drug Metab Dispos. 37(3):685–693.

Lee CM, Pohl J, Morgan ET. 2009. Dual mechanisms of CYP3A protein regulation by proinflammatory cytokines stimulation in primary hepatocyte cultures. Drug Metab Dispos. 37(4):865–872.

Li T-T, An J-X, Xu J-Y, Tuo B-G. 2019. Overview of organic anion transporters and organic anion transporter polypeptides and their roles in the liver. World J Clin Cases. 7(23):3915–3933.

Lin C-H, Hsu K-W, Chen C-H, Uang Y-S, Lin C-J. 2017. Differential changes in the pharmacokinetics of statins in collagen-induced arthritis rats. Biochem Pharmacol. 142:216–228.

Marada VVVR, Flörl S, Kühne A, Müller J, Burckhardt G, Hagos Y. 2015. Interaction of human organic anion transporter 2 (OAT2) and sodium taurocholate cotransporting polypeptide (NTCP) with antineoplastic drugs. Pharmacol Res. 91:78–87.

Mayo PR, Skeith K, Russell AS, Jamali F. 2000. Decreased dromotropic response to verapamil despite pronounced increased drug concentration in rheumatoid arthritis. Br J Clin Pharmacol. 50(6):605–613.

Ohtsuki S, Schafer O, Kawakami H, Inoue T, Liehner S, Saito A, Ishiguro N, Kishimoto W, Ludwig-Schwilling E, Ebner T, et al. 2012. Simultaneous absolute protein quantification of transporters, cytochromes P450, and UDP-glucuronosyltransferases as a novel approach for the characterization of individual human liver: comparison with mRNA levels and activities. Drug Metab Dispos. 40(1):83–92.

Ono H, Tanaka R, Suzuki Y, Oda A, Ozaki T, Tatsuta R, Maeshima K, Ishii K, Ohno K, Shibata H, et al. 2021. Factors Influencing Plasma Coproporphyrin-I Concentration as Biomarker of OATP1B Activity in Patients With Rheumatoid Arthritis. Clin Pharmacol Ther. 110(4):1096–1105.

Pavlova A, Sakurai H, Leclercq B, Beier DR, Yu AS, Nigam SK. 2000. Developmentally regulated expression of organic ion transporters NKT (OAT1), OCT1, NLT (OAT2), and RCT1. Am J Physiol Renal Physiol. 278(4):F635–F643.

Saib S, Delavenne X. 2021. Inflammation induces changes in the functional expression of P-gp, BCRP, and MRPs: An overview of different models and consequences for drug disposition. Pharmacutics. 9:28.

Schmitt C, Kuhn B, Zhang X, Kivitz AJ, Grange S. 2011. Disease-drug interaction involving tocolzumab and simvastatin in patients with rheumatoid arthritis. Clin Pharmacol Ther. 89(5):735–740.

Stanke-Labesque F, Gautier-Veyret E, Chhun S, Guilhaumou R, French Society of Pharmacology and Therapeutics 2020. Inflammation is a major regulator of drug metabolizing enzymes and transporters: Consequences for the personalization of drug treatment. Pharmacol Ther. 215:107627.

Sukhai M, Yong A, Pak A, Piquette-Miller M. 2001. Decreased expression of P-glycoprotein in interleukin-1beta and interleukin-6 treated rat hepatocytes. Inflamm Res. 50(7):362–370.

Wang H, Han Q, Chen Y, Hu G, Xing H. 2021. Novel insights into cytochrome P450 enzyme and solute carrier families in cadmium-induced liver injury of pigs. Ecotoxicol Environ Saf. 211:119110.

Wollmann BM, Syversen SW, Vistnes M, Lie E, Mehus LL, Molden E. 2018. Associations between cytokine levels and CYP3A4 phenotype in patients with rheumatoid arthritis. Drug Metab Dispos. 46(10):1384–1389.

Wu K-C, Lin C-J. 2019. The regulation of drug-metabolizing enzymes and membrane transporters by inflammation: Evidences in inflammatory diseases and age-related disorders. J Food Drug Anal. 27(1):48–59.

Yasui Y, Kudo A, Kurosaki M, Matsuda S, Muraoka M, Tamaki N, Suzuki S, Hosokawa T, Ueda K, Matsunaga K, et al. 2014. Reduced organic anion transporter expression is a risk factor for hepatocellular carcinoma in chronic hepatitis C patients: a propensity score matching study. Oncology. 86(1):53–62.