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Effects of Adjuvant-Induced Inflammation on Disposition of Diclofenac and Its Metabolites in Perfused Rat Liver

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ABSTRACT

The reactive metabolites of diclofenac (DF) such as 1-*O*-acyl glucuronide (DF-Glu) are hypothesized to result in idiosyncratic hepatotoxicity. However, it is unclear whether inflammation affects the hepatic disposition of DF and its metabolites. To clarify the alterations in the disposition of DF and its metabolites in inflammatory conditions, we performed *in situ* perfused rat liver experiments. Using adjuvant arthritis rats as a model of inflammation, the elimination of DF, 4'-hydroxydiclofenac, and DF-Glu from the perfusate was observed to be delayed in comparison with the control. Parameter sensitivity analysis for hepatic DF disposition revealed that the area under the plasma concentration-time curve (*AUC*) and the maximum concentration (C_{max}) of DF-Glu in the liver markedly increased along with a decrease in intrinsic excretion clearance of DF-Glu ($CL_{int,bile,Glu}$) and an increase in intrinsic glucuronidation clearance ($CL_{int,Glu}$) of DF-Glu. It is possible that the elimination of DF-Glu from the perfusate in adjuvant arthritis rats was delayed via reduction of biliary excretion of DF-Glu.

Introduction

Diclofenac (DF; *o*-[(2, 6-dichlorophenyl) amino] phenylacetic acid), a nonsteroidal anti-inflammatory drug, is used for the treatment of mild-to-moderate pain, fever, and inflammation.[1,2] DF is metabolized to 4'-hydroxydiclofenac (DF-4'OH) and 5-hydroxydiclofenac by cytochrome P450 (CYP)2C subfamily[3] and also conjugated to form diclofenac-1-*O*-acyl glucuronide (DF-Glu) by rat UDP-glucuronosyltransferase (UGT)2B1 and human UGT2B7.[4] Glucuronides including DF-Glu, especially in rats, are extensively excreted in the bile via multidrug resistance–associated protein 2 (Mrp2) expression in the canalicular membranes of the hepatocytes[5,6] and are effluxed in the blood via Mrp3 expression in the basolateral membranes of the hepatocytes.[7,8] DF-Glu is postulated to be a likely cause of idiosyncratic hepatotoxicity via the formation of covalent protein adducts with endogenous proteins as reactive metabolites.[9,10] Another reactive metabolite of DF is a quinone imine intermediate generated via oxidation by CYP. DF-4'OH is also known to form covalent protein adducts.[11-13]

Inflammatory conditions such as rheumatoid arthritis and Crohn's disease are characterized by a reduction in the hepatic clearance of several highly cleared drugs.[14-17] Adjuvant-induced arthritis (AA) rats have been used as an animal model for rheumatoid arthritis in the development of new anti-inflammatory drugs because they exhibit a systemic inflammatory disease with similar bone and cartilage alterations to those observed in clinical rheumatoid arthritis.[18] Changes in the pharmacokinetics and pharmacological effects of several drugs have been reported in AA rats, such as elevated plasma concentrations of cyclosporine A, acebutolol, and propranolol[15,16,19] and prolongation of sedative effects of pentobarbital.[20] We have previously demonstrated that the plasma levels of lactate dehydrogenase, aspartate aminotransferase, alkali phosphatase, albumin, and α_1-acid glycoprotein are altered in AA rats.[21] The plasma levels of lactate dehydrogenase, aspartate aminotransferase, and alkali phosphatase increased in a time-dependent manner for up to 7 days during AA induction. In the AA rats, the CYP activities and serum protein binding, which are the determining factors for the pharmacokinetics of metabolic capacity–limited drugs, are altered.[21] The levels of albumin and α_1-acid glycoprotein significantly decreased and increased, respectively, upon adjuvant dosing when compared with those of control rats.[21] We have also reported that the total clearance (CL_{tot}) of flurbiprofen in AA rats significantly increased when compared to that of controls owing to an extensive increase in the unbound fraction of flurbiprofen in the plasma, although glucuronidation activities were impaired and CYP contents were decreased.[22] In the case of metabolic capacity–limited drugs, such as DF, the increase in plasma-free fraction could act as a counterbalance to the decrease in metabolic activities.

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An *in situ* perfused rat liver system has been used to investigate liver transport and metabolism of various drugs and has several advantages for investigating drug disposition in the liver. For example, this system is useful for investigating both phase I and phase II metabolism, examining biliary excretion, and evaluating the effects of hepatic blood flow and protein binding on metabolism. To evaluate the effects of inflammation on the intrinsic metabolism of DF and bile excretion of DF and its metabolites, while avoiding the effect of changes in protein binding, we performed *in situ* perfused rat liver experiments in the absence of plasma protein in the perfusate in AA rats. The data obtained were analyzed using a semi-physiologically based pharmacokinetic (semi-PBPK) model to understand the factors determining the hepatic disposition of DF.

Materials and Methods

Ethical Approval of the Study Protocol

The study protocol was approved by the Committee for the Care and Use of Laboratory Animals at Kindai University Faculty of Pharmacy (Osaka, Japan).

Chemicals

DF and taurocholic acid (TA) were purchased from Sigma-Aldrich (St. Louis, MO). DF-Glu and DF-4'OH were obtained from Daiichi Pure Chemicals Company (Tokyo, Japan). Mefenamic acid used as an internal standard for HPLC was obtained from Yamamoto Corporation (Osaka, Japan). All other chemicals and solvents were of the highest commercially available purity or of HPLC grade.

Animals and Treatment

Seven- to 8-week-old female Sprague Dawley rats, weighing 210-250 g, were purchased from CLEA Japan (Tokyo, Japan). The rats were housed in a temperature-controlled room with free access to standard laboratory chow and water.

For AA induction, rats were immunized under light ether anesthesia by injecting into the right hindpaw and tail base as described previously.[23]

In Situ *Rat Liver Perfusions*

In situ rat liver perfusions were performed as described previously.[24] The volume of perfusion medium in all experiments was 100 mL, and the perfusate consisted of Krebs-Ringer-bicarbonate solution (pH 7.4) containing 0.18% (w/v) D-glucose, and 24 μM TA saturated with 95% O_2/5% CO_2. Rats cannulated in the bile duct, hepatic portal vein, and inferior vena cava were perfused at a flow rate of 26 mL/min for an equilibration period of 20 min with drug-free perfusate (Krebs-Ringer-bicarbonate solution with 24 μM TA). DF was added to the perfusate (final concentration: 10 μg/mL; 31.4 μM) reservoir, and the perfusate samples (1 mL) were collected from the recirculating reservoir at 0, 1, 2, 3, 5, 10, 15, 20, 30, 40, 50, and 60 min and the same volume of blank perfusion medium was immediately added to the perfusate. Bile samples were collected at 10-min intervals for up to 60 min. All samples were collected into a cooling tube containing 5 μL of 17% phosphoric acid to prevent the DF-Glu degradation. To determine the hepatic concentrations of DF, DF-Glu, and DF-4'OH, the liver was excised 20 min after initiation of perfusion using separate rats. All samples were stored at −30°C until analysis.

HPLC Determination

DF, DF-Glu, and DF-4'OH concentrations in the perfusate and bile were simultaneously measured using HPLC as described previously.[24] The liver was homogenized with 2 volumes of 0.1 M phosphate buffer (pH 4.5). A 100-μL homogenate was added to 400 μL of 0.01 M acetate buffer (pH 2.5), the 40 μL of internal standard (5 μg/mL), and 3 mL of ethyl acetate. After mixing for 10 min and centrifuging for 10 min at 2000 × *g*, the supernatant was evaporated to dryness using centrifugal evaporator (Tokyo Rikakikai Company, Tokyo, Japan) at 35°C. The residue was dissolved in 200 μL of methanol and aliquots of 20 μL were injected into the HPLC system. Standard curves for DF and DF metabolites were linear over the concentration range of 0.01-100 μg/mL ($r > 0.94$ and <10% error). The minimum quantifiable concentrations of DF and its metabolites were 0.01 μg/mL.

Pharmacokinetic Analysis

The steady-state volume of distribution (Vd_{ss}) of DF was calculated by a noncompartmental analysis using the time course of DF concentrations in the perfusate. The elimination half-life ($t_{1/2}$) of DF was determined from the slope of the terminal phase of the log (perfusate concentration) versus time profile by linear regression. The area under the plasma concentration-time curve (AUC) was calculated from 0 to 60 min ($AUC_{0\text{-}60}$) for DF, DF-Glu, and DF-4'OH in the perfusate by using the linear trapezoidal rule. The hepatic clearance (CL_h) was calculated as the DF dose divided by the $AUC_{0\text{-}60}$. Apparent biliary clearance (CL_{bile}) of DF was calculated as the cumulative biliary excreted amount of DF-Glu divided by $AUC_{0\text{-}60}$ of DF.

Model Development and Parameter Estimation

A semi-PBPK model was developed to describe the pharmacokinetics of DF, DF-Glu, and DF-4'OH and is shown in Figure 1. The model consists of perfusate and liver compartments, which are linked by a perfusion flow. For DF, the liver is described as a flow-limited, single, well-stirred compartment, and DF is assumed to be metabolized in the liver to its metabolites. Metabolism from DF to its metabolites other than DF-Glu and DF-4'OH was assumed to be negligible. For metabolites, the liver compartments were divided into 2 subcompartments linked by influx and efflux clearances (PS_{inf} and PS_{eff}), and each metabolite is assumed to be excreted into bile (DF-Glu) or further metabolized to other metabolites (DF-4'OH). The mass balance equations for DF, DF-Glu, and DF-4'OH are as follows:

DF

$$V_p \cdot \frac{dC_p}{dt} = -Q_H \cdot C_p + Q_H \cdot \frac{C_H}{K_p}$$

$$V_H \cdot \frac{dC_H}{dt} = Q_H \cdot C_p - Q_H \cdot \frac{C_H}{K_p} - \left(CL_{int,Glu} + CL_{int,OH}\right) \cdot \frac{C_H}{K_p}$$

DF-Glu