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Acute Peripheral Inflammation Increases Plasma Concentration of Hypoglycemic Agent Nateglinide with Decreased Hepatic Drug-Metabolizing Activity in Rats

Moeko Kojina, Keiichiro Suzuki, Akane Nishiwaki, and Tetsuya Aiba*

Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University;
Okayama 700–8558, Japan.

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The effects of inflammation on hypoglycemic agents were evaluated in male rats with acute peripheral inflammation (API). Nateglinide (NTG) was utilized as a model compound, since it is a hepatically-metabolized compound and its metabolism is mainly mediated by CYP 2C11 enzyme. In the experiments, rats were subjected to carrageenan injection into their hind paws for API induction, and the plasma concentration profiles of NTG were then examined. In addition, pooled liver microsomes were prepared from control and API rats, and the hepatic drug-metabolizing activity toward NTG and the hepatic expression of CYP2C11 protein were evaluated. It was shown that the plasma concentration of NTG following its intravenous administration decreases at a slower rate in API rats than that in control rats. It was also indicated in the incubation study with the liver microsomes that the hepatic drug-metabolizing activity toward NTG decreases in API rats. Additionally, it was revealed in Western immunoblotting that the hepatic expression of CYP2C11 protein decreases in API rats. These findings suggest that inflammation occurring in peripheral tissues brings about a decrease in hepatic NTG metabolism by suppressing the hepatic expression of CYP2C11 protein, causing an alteration of the plasma concentration profile of NTG with its impaired elimination.

Key words acute inflammation; CYP2C11; hepatic drug metabolism; nateglinide

INTRODUCTION

As is well-recognized, inflammation, particularly the acute form, arises with the cardinal symptoms of pain, heat, swelling, and redness.^{1,2)} This is because of the fact that the proinflammatory mediators that are released in response to noxious stimuli act immediately at the site they are released, causing vasodilation and nociception.^{1,2)} The symptoms can therefore be considered as a reflection of their local effects, and yet it may be necessary to consider that they also have a general influence affecting the whole body, in which they are probably conveyed to various tissues/organs *via* the blood circulation.

Since the liver function for handling xenobiotics is largely involved in the plasma concentration profile of therapeutic compounds, an altered liver function related to the released proinflammatory mediators probably has a non-negligible impact on the effectiveness of pharmacotherapy. In fact, it was demonstrated that the hepatic drug-metabolizing activity alters with systemic administration of a potent sepsis inducer lipopolysaccharide or with acute peripheral inflammation by subcutaneous carrageenan treatment.^{3–5)} In addition, an unexpected increase in the blood concentration of therapeutic compounds is often reported in clinical situations when patients suffer from cachexia, a complex syndrome caused by inflammatory cytokines.^{4,6,7)} Based on these findings and reports, hepatic drug metabolism may decrease under inflammatory conditions, causing altered plasma concentration profiles of therapeutic compounds. However, it should be noted that decreased hepatic drug-metabolizing activity does not always result in altered pharmacokinetics of hepatically-metabolized compounds, as exemplified by decreased hepatic expression of drug-metabolizing enzymes weakly influencing the plasma concen-

tration profile of the substrate rosiglitazone,^{8,9)} along with the report of unaltered morphine pharmacokinetics with decreased hepatic activity of glucuronidation.¹⁰⁾ Since altered plasma concentration profiles of therapeutic compounds potentially lead to failed pharmacotherapy and/or adverse events due to medication, it is worth characterizing and understanding inflammation-induced altered pharmacokinetics of therapeutic compounds, especially compounds with a narrow therapeutic window and potentially causing life-threatening adverse events.

In this study, we selected a therapeutic compound among hypoglycemic agents and evaluated its altered pharmacokinetics under inflammatory conditions, considering the fact that diabetic patients readily develop inflammation. In diabetes, microvascular diseases and immune dysfunction also readily develop,^{11,12)} which often impairs the skin barrier function, causing spontaneous healing processes to be compromised.¹²⁾ As a result, diabetic patients develop exaggerated skin ulcers with refractory bacterial/fungal infections, leading to inflammatory conditions.^{12–14)} As for pharmacotherapy with hypoglycemic agents, it is important to set an appropriate dosage for each patient to promote adequate glycemic control while avoiding the compound's adverse effects including hypoglycemia.^{15,16)} With these considerations, we examined the pharmacokinetics of the hypoglycemic compound in disease model rats with acute peripheral inflammation, which revealed greater risks of drug events due to an increased plasma concentration of the compound caused by impaired hepatic drug metabolism.

MATERIALS AND METHODS

Materials Nateglinide (NTG) was purchased from Tokyo Chemical Industry (Tokyo, Japan). It was used in this study as

*To whom correspondence should be addressed. e-mail: taiba@okayama-u.ac.jp

a representative compound of hypoglycemic agents in clinical use. λ -Carrageenan was bought from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Sulfaphenazole and ketoconazole were obtained from Cayman Chemical (Ann Arbor, MI, U.S.A.) and Tokyo Chemical Industry, respectively. β -Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was from Oriental Yeast (Tokyo, Japan). Anti-rat α_1 -acid glycoprotein (AGP) polyclonal antibody from rabbits (catalogue No. 18130) was obtained from Life Diagnostics Inc. (West Chester, PA, U.S.A.). Anti-CYP 2C11 polyclonal antibody (ab3571) from rabbits was purchased from Abcam (Cambridge, U.K.). All other chemicals and materials were of the highest or finest grade available from local distributors.

Animals Male Wistar rats at 7–8 weeks old (230–340 g) were purchased from Japan Charles River (Yokohama, Japan). They were caged in an air-conditioned room with the temperature and relative humidity controlled at 23–24°C and 40–50%, respectively. The rats were allowed free access to a standard laboratory diet and water. The 12-h daylight time was set from 8:00 a.m. to 8:00 p.m. All animal experiments were approved by the institutional animal ethics committee (OKU-2015421/OKU-2018141), and performed in accordance with the guidelines for animal experimentation of Okayama University.

In the rats randomly selected 24 h before the experiments, API was induced by a subcutaneous administration of λ -carrageenan to their hind paws at a dose of 64 mg/kg.⁵⁾ They were then placed back in their cage with free access to the diet and water. A successful induction of API was confirmed with an increased plasma AGP level by performing Western immunoblotting, as described later,^{17,18)} as AGP is known as one of the acute phase proteins whose plasma levels increase in the presence of inflammation. The rats not selected were supplied for the experiments as control rats without any further treatments.

Evaluation of Plasma Concentration Profile of NTG in Control and API Rats Under anesthesia with pentobarbital (80 mg/kg, intraperitoneally (i.p.)), each rat was fixed on its back, and heparinized polyethylene tubing (SP-31, Natsume Seisakusho, Tokyo, Japan) was then inserted into the right femoral artery for blood collection.⁵⁾ Then, the NTG solution was administered into the left femoral vein at a dose of 12 mg/kg using a 1-mL syringe equipped with a 27-gauge needle. While NTG is orally administered in clinical situation and its intestinal absorption is nearly complete,^{19,20)} the relationship between the plasma concentration profile of NTG and its hepatic metabolism can be readily evaluated by characterizing the plasma profile of NTG following its intravenous administration. Regarding the NTG dose used in this study, it was chosen by referring to literatures,^{19,21)} considering that the plasma concentration of NTG is well determined even for the specimen collected in the elimination phase with our determination method described later, although it was inevitably higher as compared to that applied in clinical use (270–360 mg/d, t.i.d.). The NTG solution was prepared by dissolving NTG into dimethyl sulfoxide at a concentration of 150 mg/mL. Following the administration, 250 μ L of blood was drawn from the right femoral artery at 2, 7, 12, 20, 40, 60, and 90 min. After these blood drawings were completed, the rat was put under deep anesthesia to be sacrificed by blood withdrawal from the inferior vena cava with a 10-mL syringe. The obtained blood samples were then centrifuged at 2000 $\times g$ for 10 min at 4°C to collect

plasma specimens, and the specimens were stored at –20°C until used for NTG determination and Western immunoblotting to evaluate the plasma AGP level. During the experiment, each rat was placed on a thermal plate kept warm at 37°C. The rat received 250 μ L of 5% glucose solution as a body fluid supplement each time the blood draw was performed.

The plasma concentration profile of NTG was examined in a model-independent manner. That is, the profile was first characterized with the following equation, and parameters A , α , B , and β in the equation were estimated by applying a curve-fit calculation to the profile²²⁾:

$$Cp(t) = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \quad (1)$$

where $Cp(t)$ designates the plasma concentration of NTG at collection time-point t . After that, the area under the curve (AUC) of the plasma NTG concentration profile and other parameters regarding the NTG pharmacokinetics were obtained with the equations below:

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \quad (2)$$

$$CL_t = \frac{D}{AUC} \quad (3)$$

$$V_{dss} = \frac{D}{\beta \cdot AUC} \quad (4)$$

where CL_t and V_{dss} designate the total body clearance of NTG and its volume of distribution, respectively, in which the elimination rate constant of NTG in the elimination phase is indicated as β . The dosed amount of NTG is denoted as D . If necessary, the elimination half-time of NTG in the elimination phase ($T_{1/2\beta}$) was calculated by dividing the natural logarithm of 2 by the parameter β . We did not evaluate the plasma concentration profile of NTG metabolites in this study. It is known that NTG is mostly metabolized to 1- and 2-hydroxyisopropyl forms, and that these metabolites also have a hypoglycemic potency.²⁰⁾ However, their plasma concentrations are reported to be roughly 1/20 of the NTG concentration,²³⁾ being negligible from the viewpoint of the potential risk due to altered plasma NTG concentration under inflammatory conditions.

Evaluation of Plasma Unbound Fraction of NTG in Control and API Rats With the plasma specimen collected from the blood sample of the inferior vena cava, the NTG test solutions were prepared. The NTG concentration in the solutions was in a range of 20–60 mg/mL (approx. 60–190 μ M), the exact values of which were later determined. The plasma unbound fraction of NTG was examined with an ultrafiltration method by utilizing a centrifugal device (Nanosep® 10K Omega™ Membrane, NIPPON Genetics, Tokyo, Japan).¹⁷⁾ In brief, 500 μ L of the NTG test solution was applied to the centrifugal device, and then the device was subjected to a 20-min centrifugation at ambient temperature. The centrifugation was performed at 1000 $\times g$ in order to filter about 10% of the applied solution. After the centrifugation, the filtered fraction was weighed, and its NTG concentration was determined as well as that of the applied solution. The plasma unbound fraction of NTG was obtained by dividing the NTG amount in the filtered fraction by the amount in the applied solution.

Evaluation of Hepatic Drug-Metabolizing Activity toward NTG in Liver Microsomes Prepared from Control and API Rats The hepatic drug-metabolizing activity to-

ward NTG was examined in pooled liver microsomes from control and API rats. With livers freshly procured from 5 rats, the microsomes were prepared in a conventional manner with ultra-centrifugation, as previously reported.²⁴⁾ During the liver procurement, blood was drawn from the inferior vena cava to collect the plasma specimen to be used in the above-mentioned assessments of the plasma AGP level and the plasma unbound fraction of NTG. The protein content of the prepared microsomes was determined with a commercially available protein assay kit (Bio-Rad, Hercules, CA, U.S.A.). They were stored at -80°C until use.

The hepatic drug-metabolizing activity was evaluated based on the β -NADPH-dependent elimination rate of NTG measured in the incubation study.^{18,25)} The incubation was performed in 1 mL of incubation mixture prepared based on isotonic phosphate buffer (pH 7.4) containing 500 μg of the microsomal protein. NTG was dissolved into the mixture at final concentrations of 10, 30, 60, and 100 μM . Each set of the incubation experiment was carried out with these 4 NTG concentrations, and at each concentration, the elimination rate of NTG was evaluated in triplicate. Following a 5-min pre-incubation period, the metabolic reaction was initiated by spiking the incubation mixture with 50 μL of 20 mM β -NADPH solution, and the incubation was carried out at 37°C . After a 20-min incubation period, the metabolic reaction was terminated by adding 500 μL of 1 M hydrochloride solution to the incubation mixture. The mixture was then agitated and chilled in an ice bath. After that, the mixture was supplied for the NTG determination described later. The incubation experiments were also performed by spiking with potassium phosphate buffer rather than β -NADPH solution to assess non-specific NTG elimination.

In each set of the incubation experiments, the β -NADPH-dependent elimination rate (v) of NTG was characterized by performing non-linear least square calculation with the Michaelis–Menten equation below^{18,25)}:

$$v = \frac{V_{\max} \cdot C}{K_m + C} \quad (5)$$

where C is the NTG concentration of the incubation mixture. $V_{\max}$ is the maximum elimination rate of NTG, and K_m is the apparent Michaelis–Menten constant regarding the NTG elimination. The intrinsic clearance (CL_{int}) was calculated by dividing $V_{\max}$ by K_m .

In addition, the hepatic drug-metabolizing activity toward NTG was examined with the liver microsomes from the control rats in the presence and absence of the CYP inhibitors sulfaphenazole and ketoconazole. Sulfaphenazole was dissolved into the incubation mixture at final concentrations of 10 and 100 μM , while ketoconazole was applied in the mixture at a final concentration of 1 μM . These concentrations of the inhibitors were set to selectively inhibit the hepatic drug-metabolizing activity *via* CYP2C and 3A isoenzymes in rats.²⁶⁾ The incubation experiments were carried out in the same manner as described above with the final concentration of NTG being set at 100 μM , and the β -NADPH-dependent elimination rates of NTG were evaluated. The elimination rates with and without the inhibitors were compared to examine whether the inhibitors affect the hepatic NTG elimination.

Analytical Method for NTG Determination NTG was determined by HPLC equipped with an octyl silica column (5 μm , 4.6×150 mm, Inertsil[®] C8-3, GL Science, Tokyo,

Japan). For the determination, 100 μL of plasma specimen was acidified by adding 1 mL of 1 M hydrochloride solution. It was mixed well, and then 6 mL of ice-cold diethyl ether was added to it. In the case of the aforementioned incubation mixture from the incubation study, 6 mL of ice-cold diethyl ether was immediately added to it. These specimens and mixtures were then vigorously agitated for 5 min, and they were centrifuged at $2000 \times g$ for 10 min at 4°C . After that, 5 out of 6 mL of the diethyl ether layer was collected to be evaporated to dryness. The residue was dissolved with 50 μL of 50% methanol to be applied to HPLC. The mobile phase for NTG determination was prepared with 72% methanol and 28% sodium phosphate buffer (10 mM, pH 2.0), and it was used at a flow rate of 1.0 mL/min. The NTG detection was performed with a spectrophotometer at a wavelength of 210 nm.

Semiquantitative Evaluation of Plasma AGP Level and Hepatic CYP2C11 Expression with Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Western Immunoblotting in Control and API Rats

The plasma AGP level and hepatic expression of CYP2C11 protein were examined with SDS-PAGE followed by Western immunoblotting in a semiquantitative manner. For the evaluation of the AGP level, electrophoresis was performed with test plasma samples prepared from the plasma specimens, and they were applied to an SDS-PAGE (15%) at 1 μg of plasma protein/lane.¹⁸⁾ For the evaluation of CYP2C11 expression, the test samples prepared from hepatic microsomes were applied to a polyacrylamide gel (10% Mini-PROTEAN[®] TGX[™] precast gel, Bio-Rad) at 1 μg of microsomal protein/lane. Following the electrophoresis, the migrated proteins were transferred to a nitrocellulose membrane, and then Western immunoblotting was carried out, in which the anti-AGP antibody was diluted at 1:5000, while the anti-CYP2C11 antibody was diluted at 1:2000 for use. The migrated proteins on the nitrocellulose membrane were visualized with a commercially-available stain kit (Vectastain[®] Elite ABC Kit, Vector Laboratories, Burlingame, CA, U.S.A.) and DAB substrate kit (Vector Laboratories). The migration pattern visualized on the nitrocellulose membrane was densitometrically processed in a semiquantitative manner.^{18,25)} In the electrophoresis and Western immunoblotting, the specimens from control and API rats were simultaneously handled. The values in the API rats are evaluated as percentages of those in control rats (100%).

Data Analysis For values considered to be normally distributed, they are shown as means $\pm$ standard error (S.E.), and the differences between two groups were examined by Student's t -test with $p < 0.05$ being considered significant. If it was not the case, values were handled in a non-parametric manner,²⁷⁾ in which they were summarized with the median and quartiles. These values are expressed by employing a box-and-whisker plot, where the first and third quartiles are the bottom and top of the box, respectively. The whisker beneath the box corresponds to the range from the first quartile to the lowest data point or to the point given by subtracting 1.5 times the value of the interquartile from the first quartile, whichever is shorter. The whisker above the box indicates the range from the third quartile to the highest data point or to the point given by adding 1.5 times the value of the interquartile to the third quartile, whichever is shorter. The differences between the two groups were evaluated by the Mann–Whitney U -test, and $p < 0.05$ was considered significant.

RESULTS

Evaluation of Plasma AGP Levels in Rats with Carrageenan Treatment The plasma AGP levels in rats were semi-quantitatively evaluated with Western immunoblotting, and it was checked whether API was successfully induced with the carrageenan treatment. It was indicated that the plasma AGP levels in rats with the treatment noticeably increased to be about 2 times as high as those in rats without the treatment, reflecting the fact that API was appropriately induced with the treatment (Fig. 1).

Fig. 1. Increased Plasma AGP Levels in Rats with API Induced by Carrageenan Treatment

The AGP levels were semi-quantitatively determined with Western immunoblotting. A representative result of immunoblotting is shown on the upper side of the figure, in which the visualized migrated protein corresponding to the molecular weight of AGP is indicated with a closed triangle. Data are shown using a box-and-whisker plot with medians from 6 measurements. The median is indicated as a bar in the box. * $p < 0.05$: significantly different from the control value.

Evaluation of the Plasma NTG Profiles in Control and API Rats The plasma concentration profiles of NTG following its intravenous administration were examined in control and API rats. As shown in Fig. 2, their plasma NTG concentrations similarly receded in a biphasic manner, while the rate of receding was slower in API rats than that in control rats. When these profiles were characterized, it was revealed that the CL_t value of NTG in API rats decreased by 35% from the value in control rats, and that the V_{dss} value in API rats increased by about 1.6 times compared with the control value (Table 1).

For a potential factor influencing the NTG pharmacokinetics in API rats, the plasma unbound fraction of NTG was evaluated in control and API rats. The unbound fraction in control rats was calculated to be $0.70 \pm 0.11\%$, while that in API rats was $0.88 \pm 0.04\%$. The difference in the unbound fractions between control and API rats did not reach significance, although the value in API rats was slightly larger than that in control rats.

Evaluation of Hepatic NTG Metabolism in Control and API Rats The hepatic drug-metabolizing activity toward NTG in control and API rats was then evaluated in the incubation study with liver microsomes. As shown in Fig. 3, the hepatic elimination rates of NTG varied in a concentration-dependent manner, and the rates were generally slower in API rats as compared with those in control rats. When the elimina-

Table 1. Pharmacokinetic Parameters Calculated Based on the Plasma NTG Concentration–Time Profiles in Control and API Rats^{a)}

Parameters	Control rats	API rats
$AUC^{b)}$	726.0 ± 6.4	$1133.0 \pm 58.0^{f)}$
$CL_t^{c)}$	16.5 ± 0.2	$10.7 \pm 0.5^{f)}$
$V_{dss}^{d)}$	431.7 ± 2.6	$685.7 \pm 30.9^{f)}$
$T_{1/2\beta}^{e)}$	18.1 ± 0.2	$44.4 \pm 0.2^{f)}$

a) Data are shown as the mean $\pm$ standard error (S.E.) from 5–6 experiments. b) Data are expressed in units of $\mu\text{g} \cdot \text{min}/\text{mL}$. c) Data are expressed in units of $\text{mL}/\text{min}/\text{kg}$ of body weight. d) Data are expressed in units of mL/kg of body weight. e) Data are expressed in units of min. f) $p < 0.05$, significantly different from the corresponding value in the control rats.

Fig. 2. The Plasma Concentration–Time Profiles of NTG in 5 Control (Panel A) and 6 API (Panel B) Rats

NTG was intravenously administered at a dose of $12 \text{ mg}/\text{kg}$. In each rat, the plasma NTG concentration profile was analyzed with a curve-fitting calculation to determine its pharmacokinetic parameters. In both panels, all data points obtained in the experiments are presented, and the plasma NTG concentration profile calculated with mean values of the pharmacokinetic parameters is shown with a solid curve. The plasma profile for each rat is indicated with a light gray dotted line. The mean values of the pharmacokinetic parameters were significantly different between control and API rats ($p < 0.05$, see Table 1).

Fig. 3. The Michaelis–Menten Profiles Regarding Hepatic NTG Elimination in the Incubation Study

The incubation study was performed with liver microsomes prepared from control and API rats. Data from 4 experiments for control rats and 4 experiments for API rats are shown in closed light gray and dark gray circles, respectively. In each experiment, the elimination profile was analyzed with a curve-fitting calculation to determine Michaelis–Menten parameters. The best-fit curve obtained in each experiment is indicated with a dotted light gray line. The NTG elimination profiles calculated with mean values of the Michaelis–Menten parameters for control and API rats are shown in solid dark gray and light gray lines, respectively. The Michaelis–Menten parameters were significantly different between control and API rats ($p < 0.05$, see Table 2).

Table 2. Michaelis–Menten Parameters for NTG Metabolism Determined in Liver Microsomes Prepared from Control and API Rats^{a)}

Parameters	Control rats	API rats
K_m ^{b)}	55.51 ± 1.04	$53.17 \pm 0.25^e)$
V_{max} ^{c)}	3.20 ± 0.03	$2.19 \pm 0.11^e)$
CL_{int} ^{d)}	57.75 ± 1.61	$41.14 \pm 2.34^e)$

a) Data are shown as the mean $\pm$ S.E. from 4 sets of the incubation experiments. b) Data are expressed in units of μM . c) Data are expressed in units of nmol/min/mg of microsome protein. d) Data are expressed in units of $\mu L/min/mg$ of microsome protein. e) $p < 0.05$, significantly different from the corresponding value in the control rats.

tion rate was characterized with the Michaelis–Menten equation, it was indicated that the V_{max} value in API rats decreased by 32% from that in control rats, and that the K_m value in API rats slightly but significantly altered as compared with that in control rats (Table 2). These altered parameters resulted in a noticeable decrease in the CL_{int} value in API rats (Table 2), indicating that the hepatic drug-metabolizing activity toward NTG was compromised in API rats.

The hepatic drug-metabolizing activity toward NTG was also examined in the presence of inhibitors that selectively inhibit CYP2C and 3A isoenzymes. It was shown that the NTG elimination rate was noticeably reduced when sulfaphenazole was applied at a final concentration of $100 \mu M$, while it was not noticeably affected when sulfaphenazole was applied at $10 \mu M$ (Table 3). The observed decrease in the NTG elimination rate with ketoconazole did not reach significance (Table 3).

Evaluation of the Hepatic Expression of CYP2C11 Protein in Control and API Rats The hepatic expression of CYP2C11 protein was evaluated in control and API rats with Western immunoblotting in a semi-quantitative manner. As a result, the protein expression in API rats was shown to significantly decrease by 37% as compared with that in control rats (Fig. 4).

Table 3. NTG Metabolic Rate Determined with or without Inhibitors in Liver Microsomes from Control Rats^{a)}

Substrate ^{c)}	Inhibitor	Conc. of inhibitor ^{d)}	Metabolic rate of NTG ^{b)}	
			without inhibitor	with inhibitor
NTG	Sulfaphenazole	100	2.58 ± 0.11	$1.36 \pm 0.15^e)$
NTG	Sulfaphenazole	10	2.86 ± 0.22	2.58 ± 0.16
NTG	Ketoconazole	1	2.32 ± 0.32	1.92 ± 0.02

a) Data are shown as the mean $\pm$ S.E. from 3–4 experiments. b) Data are expressed in units of nmol/min/mg of microsome protein. c) NTG was used at a final concentration of $100 \mu M$. d) Final concentrations of inhibitor used in the experiment are expressed in units of μM . e) $p < 0.05$, significantly different between the values with and without inhibitor.

Fig. 4. The Hepatic Expression of CYP2C11 Protein Evaluated with Western Immunoblotting in Control and API Rats

A representative result of Western immunoblotting is shown on the upper side of the figure. The visualized migrated protein corresponding to the molecular weight of CYP2C11 is indicated with a closed triangle. Data are shown using a box-and-whisker plot with medians from 9–10 measurements. The median is indicated as a bar in the box. * $p < 0.05$; significantly different from the control value.

DISCUSSION

In diabetic patients who are receiving pharmacotherapy, it is important to perform careful dosage management of the hypoglycemic agent, since it will result in the blood sugar being appropriately controlled, which ensures not only the prevention of complications associated with hyperglycemia, but also avoidance of episodes of hypoglycemia.^{15,16)} However, the plasma concentrations of therapeutic agents are disrupted under some clinical conditions, hindering the effective performance of pharmacotherapy.⁶⁾ To perform effective pharmacotherapy, it is therefore necessary to understand underlying factors responsible for the disruption. In this study, we focused on the altered pharmacokinetics of the hypoglycemic agent with inflammation, since patients with diabetes are known to be prone to inflammatory complications,^{11,12)} in which the hepatic drug-metabolizing activity is affected, having a non-negligible impact on the blood concentrations of compounds.

As shown in Fig. 2, it was demonstrated that the plasma concentration profiles of NTG alters and NTG elimination was noticeably retarded in API rats. As a result, the CL_t value in API rats decreases from the control value (Table 1). The V_{dss} value was also shown to be affected, noticeably increasing in API rats (Table 1). This is probably due to the fact that the

vasodilation and increased permeability of microvessels occurring under inflammatory conditions facilitate NTG distributing to the extravascular fluid and tissues. On the other hand, the plasma unbound fraction of NTG in API rats was shown to be comparable with that in control rats, suggesting that the decreased CL_t value reflects a decrease in the hepatic drug-metabolizing activity toward NTG in API rats. As expected, in the incubation study subsequently performed with liver microsomes, the NADPH-dependent elimination rates of NTG in API rats were shown to be slower than those in control rats (Fig. 3), rendering the CL_{int} value smaller in API rats as compared with that in control rats (Table 2). These results indicate that the intrinsic activity regarding hepatic NTG metabolism is reduced in API rats. Additionally, it was demonstrated that the NTG elimination is suppressed with sulfaphenazole applied at a higher concentration, while the elimination remains unaltered in the presence of ketoconazole (Table 3). Since sulfaphenazole has been reported to inhibit CYP2C11-mediated hepatic drug metabolism in a selective manner when applied at a concentration of $100\mu\text{M}$,²⁶⁾ it is considered that NTG metabolism is mainly mediated by the CYP2C11 isozyme, and to a lesser degree by the CYP3A2 isozyme, in our incubation experiments.²⁸⁾ Along with these findings and the understanding that the CYP2C11 isozyme is predominantly expressed among the CYP2C family in male rat liver,²⁹⁾ we examined the hepatic expression of CYP2C11 protein, and observed that it appears to decrease in API rats (Fig. 4). Therefore, it can be concluded that the hepatic drug-metabolizing activity toward NTG decreases with a decreased hepatic expression of the CYP2C11 isozyme in API rats, being reflected in the altered plasma concentration profile of NTG with its impaired elimination. Since it was previously demonstrated that the hepatic expression of CYP3A2 protein noticeably decreases in API rats, being a cause for decreased hepatic drug-metabolizing activity toward the CYP3A substrate midazolam,⁵⁾ it is rationally assumed that the altered plasma concentration profile of NTG in API rats is also related to the decreased hepatic expression of CYP3A2 protein. Besides the hepatic drug-metabolizing enzymes, the hepatic transporters OATP1B1 and IB3 are known to be involved in the plasma concentration profile of NTG to some extent in patients.^{30,31)} We did not currently evaluate an involvement of those transporters in the altered plasma concentration profile of NTG, but it will be necessary to fully clarify the mechanism responsible for the pharmacokinetics and disposition of NTG in API rats.

As for mechanisms responsible for the inflammation-related decrease in the hepatic expression of the CYP2C11 isozyme, the *CYP2C11* gene being transactivated by the pregnane X receptor (PXR/NR1I2) may be involved,³²⁾ in addition to the fact that the nuclear factor-kappaB (NF- κ B) is activated by proinflammatory mediators, such as interleukin-1 β and -6.^{33,34)} The proinflammatory mediators facilitate a dissociation of NF- κ B from the inhibition factor inhibitor of kappaB (I κ B), releasing NF- κ B to be activated,^{35,36)} and the activated NF- κ B then functions against PXR, preventing it from forming a heterodimer with the retinoid X receptor alpha (RXR α /NR2B1).³⁷⁾ This results in the suppression of *CYP2C11* gene expression, since the heterodimer plays a significant role in the PXR-induced gene transactivation process.³⁸⁾ Regarding the inhibition process mediated by NF- κ B, it was also reported that NF- κ B interacts with the promoter region of the *CYP2C11* gene, obstructing

the association of the PXR-RXR heterodimer with the region for initiating transactivation.^{39,40)} As a consequence of these processes and interactions, hepatic *CYP2C11* gene expression decreases under inflammatory conditions, and the hepatic expression of CYP2C11 protein is reduced.⁴¹⁾

In this study, we revealed that the plasma concentration profile of NTG alters under inflammatory conditions, due to the fact that the hepatic drug-metabolizing activity decreases with inflammation. This means that a blood sugar level that has been well-managed so far by taking a hypoglycemic agent may unexpectedly become unstable if the patient develops an inflammatory condition. Although it is necessary to further clarify whether the efficacy of hypoglycemic agents is affected by inflammation,⁴²⁾ the current findings provide an advisory regarding the potential risk of pharmacotherapy using compounds with a narrow therapeutic concentration window. For patients with inflammatory disorders, additional attention is required in order to perform pharmacotherapy effectively.

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Conflict of Interest The authors declare no conflict of interest.

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