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Contribution of organic anion transporting polypeptide (OATP) 1B1 and OATP1B3 to hepatic uptake of nateglinide, and the prediction of drug–drug interactions via these transporters

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drug–drug interaction; nateglinide; OATP1B3; organic anion transporting polypeptide (OATP) 1B1

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Abstract

Objectives We have investigated the contributions of organic anion transporting polypeptide (OATP) 1B1 and OATP1B3 to the hepatic uptake of nateglinide, and the possibility of drug–drug interactions via these transporters.

Methods Uptake studies using transporter-expressing HEK293 cells and cryopreserved human hepatocytes were performed to examine the contributions of each transporter. Inhibition studies using cryopreserved human hepatocytes were performed to examine the possibility of drug–drug interactions.

Key findings The rate of saturable hepatic uptake of nateglinide using human hepatocytes was 47.6%. A certain increase in uptake was observed in the examination using transporter-expressing HEK293 cells, indicating contributions of OATP1B1 and OATP1B3 to hepatic nateglinide uptake. The 50% inhibitory concentration (IC50) values of nateglinide using cryopreserved human hepatocytes for uptake of estrone 3-sulfate (substrate of OATP1B1), and cholecystokinin octapeptide (substrate of OATP1B3) were 168 and 17.4 μmol/l, respectively. Moreover, ciclosporin inhibited saturable hepatic uptake of nateglinide with an IC50 value of 6.05 μmol/l. The calculated $1 + I_{in,max,u}$/IC50 values for inhibition of OATP1B1 and OATP1B3 by nateglinide, and the inhibition of saturable uptake of nateglinide by ciclosporin, were all close to 1, indicating a low clinical risk of drug–drug interaction with nateglinide taken up via OATP1B1 and OATP1B3.

Conclusions OATP1B1 and OATP1B3 may have contributed to the hepatic uptake of nateglinide, but the possibility of drug–drug interactions appeared to be low.

Introduction

Nateglinide is among the immediate short-acting insulinotropic agents, and facilitates postprandial insulin secretion and controls postprandial hyperglycaemia with preprandial administration.[1–3] Nateglinide is known to be metabolized in the liver, mainly by cytochrome P450 (CYP) 2C9, which is the main metabolic enzyme.[3] Therefore, the possibility of drug–drug interactions via CYP must be considered before using nateglinide. However, we have already reported that the possibility of drug–drug interactions due to inhibition of CYP by nateglinide is considered to be low, according to the results of in-vitro experiments.[4] Moreover, the possibility of drug–drug interactions between nateglinide and other drugs was examined by in-vitro experimentation using 18 compounds. We found nateglinide metabolism to be minimally affected by these other drugs, except for miconazole and fluconazole, which are potent inhibitors of multiple CYP isoforms.[5] On the other hand, repaglinide, a glinide drug similar to nateglinide, is metabolized mainly by CYP2C8 and CYP3A4, and organic anion transporting polypeptide (OATP) 1B1 is reportedly the uptake transporter in the liver contributing to the pharmacokinetics of this drug.[6,7] OATP1B1 is an uptake transporter expressed on the sinusoidal membranes of human hepatocytes, as is OATP1B3.[8–12] Furthermore, the area under the curve (*AUC*) of repaglinide reportedly rose to 244% with concomitant administration of ciclosporin exerting an inhibitory effect on CYP3A4 and numerous other transporters including OATP1B1, and the reason for this is the possibility of the inhibition of not only

CYP3A4 but also OATP1B1.[13] Based on these observations, repaglinide is widely recognized as a substrate for OATP1B1. The fact that a transporter contributes to the pharmacokinetics of this drug indicates that a drug–drug interaction via the transporter may occur. Therefore, it is important to examine the contributions of transporters and the possibility of drug–drug interactions.[14–16] On the other hand, detailed findings on hepatic uptake transporters are not available for nateglinide. In this study, we have investigated the contributions of OATP1B1 and OATP1B3 to hepatic uptake of nateglinide. The possibility of drug–drug interactions via these transporters was studied using transporter-expressing HEK293 cells and cryopreserved human hepatocytes. This is the first study in which this content has been evaluated using in-vitro systems.

Materials and Methods

Chemicals and reagents

Nateglinide was synthesized at Ajinomoto Co., Inc. (Tokyo, Japan). [^{14}C]Nateglinide was synthesized at Daiichi Pure Chemical Co. (Tokyo, Japan). The specific radioactivity was 3.22 MBq/mg, and radiochemical purity exceeded 96%. Other chemicals were obtained from the following sources: repaglinide, estrone 3-sulfate (E-sul), cholecystokinin octapeptide (CCK-8), estradiol 17β-D-glucuronide ($E_2$17βG) and rifampicin were from Sigma Chemical Co. (St Louis, MO, USA); ciclosporin (cyclosporine) was from ALEXIS Biochemicals (San Diego, CA, USA); [^{3}H]$E_2$17βG (1.69 TBq/mmol), [^{3}H]E-sul (2.01 TBq/mmol) and [^{3}H]CCK-8 (2.59 TBq/mmol) were from Perkin Elmer Japan Co., Ltd. (Kanagawa, Japan). All other chemicals used were of HPLC grade or the highest purity commercially available.

Uptake and inhibition study using cryopreserved human hepatocytes

This experiment was performed based on a method described previously.[17,18] Cryopreserved human hepatocytes were obtained from BD Gentest Co, Ltd. (Woburn, MA, USA). We chose four lots of cryopreserved human hepatocytes (Lots 4535498002, 4525010001, 4550908002, 4547141002) showing relatively high OATP activity. Immediately before the experiment, the hepatocytes were thawed at 37°C, quickly suspended in 10 ml ice-cold Krebs–Henseleit buffer (in mmol/l: 118 NaCl, 23.8 $NaHCO_3$, 4.8 KCl, 1.0 KH_2PO_4, 1.2 $MgSO_4{\cdot}7H_2O$, 12.5 HEPES, 1.5 $CaCl_2$, 5.0 glucose, pH 7.4 with 1 mol/l KOH), and stirred gently for washing. After centrifugation (50*g*) for 2 min at 4°C, the supernatant was removed. This procedure was repeated two more times for washing, and the cells were then resuspended in ice-cold Krebs–Henseleit buffer to yield a cell density of 2×10^6 viable cells/ml. Before the uptake studies, the cell suspensions were pre-incubated at 37°C for 3 min. The uptake studies were initiated by adding an equal volume of buffer containing substrate (or substrate and inhibitor) to the cell suspension (final cell density was 1×10^6 viable cells/ml.). After incubation at 37°C for 0.5 or 2 min, the reaction was terminated by separating the cells from the buffer. For this purpose, a part of the incubation mixture was collected and placed in a centrifuge tube containing 100 µl 2 mol/l NaOH under a layer of 400 µl oil (density, approximately 1; a mixture of silicone oil and mineral oil; Sigma-Aldrich), and the sample tube was subsequently centrifuged for 1 min at 15 000*g*. During this process, hepatocytes passed through the oil layer into the alkaline solution. After an overnight incubation at 70°C in alkali to dissolve the hepatocytes, the alkali layer only was collected in a scintillation vial. The dissolved cells were mixed with scintillation fluid, and radioactivity was measured in a liquid scintillation counter.

Uptake studies using OATP1B1- and OATP1B3-expressing HEK293 cells

OATP1B1

In this study, only the uptake study using OATP1B1-expressing HEK293 was performed at ADME & Tox. Research Institute, Sekisui Medical Co., Ltd. (Ibaraki, Japan). OATP1B1-expressing HEK293 cells and vector-transfected control cells (control cells), constructed by ADME & Tox. Research Institute, were used. Immediately before the study, culture medium was removed from a 24-well cell cultured plate, and HBSS-HEPES (in mmol/l: 1.26 $CaCl_2$, 5.4 KCl, 0.44 KH_2PO_4, 0.49 $MgCl_2{\cdot}6H_2O$, 0.41 $MgSO_4{\cdot}7H_2O$, 137 NaCl, 0.34 $Na_2HPO_4{\cdot}7H_2O$, 5.6 D-glucose, 4.2 $NaHCO_3$, 10 HEPES, pH 7.4 with 1 mol/l NaOH) was added for washing. HBSS-HEPES was removed, and fresh HBSS-HEPES (0.3 ml) was added, followed by pre-incubation for 15 min. HBSS-HEPES used for pre-incubation was removed and uptake was initiated by adding HBSS-HEPES containing substrate. After having reacted for 0.5, 2, 5 or 10 min at 37°C, uptake was terminated by adding 1 ml ice-cold phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin after immediate removal of the HBSS-HEPES used for uptake. Ice-cold PBS containing 0.2% bovine serum albumin was then removed. The same handling was repeated twice using ice-cold PBS. Cells were then solubilized in 500 µl 0.1 mol/l NaOH. The dissolved part of the cells was used for protein quantitation by the BCA protein assay method using a BCA protein assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA).[19] The other part was collected in scintillation vials and mixed with a scintillator to measure radioactivity with a liquid scintillation counter.

OATP1B3

The OATP1B3-expressing HEK293 cells (GenoMembrane, Inc., Kanagawa, Japan) and control cells (Geno Membrane, Inc.) were purchased in 12-well plates. Immediately before the study, culture medium was removed from the OATP1B3-expressing HEK293 and control cell plates, and 1 ml transport medium (in mmol/l: 125 NaCl, 4.8 KCl, 5.6 D-glucose, 1.2 $CaCl_2{\cdot}2H_2O$, 1.2 KH_2PO_4, 1.2 $MgSO_4{\cdot}7H_2O$, 25 HEPES, pH 7.4) was added and lightly shaken for washing.[20] Transport medium used for washing was removed, and fresh transport medium (1 ml) was added, followed by pre-incubation for 5 min. Transport medium used for pre-incubation was removed and uptake was initiated by adding transport medium containing substrate. After having reacted for 0.5, 2, 5 or 10 min at 37°C, the uptake was terminated by adding 1 ml ice-cold transport medium after immediate removal of the transport medium used for uptake. Cell plates were washed by shaking lightly and then removed. The same handling was repeated twice using ice-cold transport medium. Cells were then solubilized in 300 µl 2 mol/l NaOH for ≥15 min, and then 300 µl 2 mol/l HCl were added followed by shaking. The dissolved part of the cells was used for protein quantitation by the method of Lowry *et al*.[21] using a DC protein assay kit (Takara Bio Inc. Shiga, Japan), and the part collected in scintillation vials and mixed with a scintillator was used to measure radioactivity with a liquid scintillation counter.

Kinetic analyses

From the uptake study of nateglinide using cryopreserved human hepatocytes, the uptake velocity (pmol/min/10^6 cells) of nateglinide at each concentration was calculated. The uptake velocity was calculated as the velocity from 0.5 to 2 min considering the influence of adsorption to the cell surface. Kinetic parameters were obtained using nonlinear least-squares data fitting from the following equation 1:[17,22,23]

$$v = \frac{V_{max} \times S}{K_m + S} + P_{dif} \times S \quad (1)$$

Where v is the uptake velocity of nateglinide (pmol/min/10^6 cells), S is the nateglinide concentration in the reaction mixture (µmol/l), K_m is the Michaelis constant (µmol/l), V_{max} is the maximum uptake rate (pmol/min/10^6 cells), and P_{dif} is the nonsaturable uptake clearance (µl/min/10^6 cells). Fitting was performed with the Damping Gauss-Newton method using a MULTI program.[24] Simulation was performed using the obtained parameters as in the following equations 2 and 3 to estimate saturable and nonsaturable uptakes:

$$v_{saturable} = \frac{V_{max} \times S}{K_m + S} \quad (2)$$

$$v_{nonsaturable} = P_{dif} \times S \quad (3)$$

The contribution rate of saturable hepatic uptake was calculated by the following equation 4:

$$\text{The contribution rate of saturable hepatic uptake}\,(\%) = \frac{V_{max}/K_m}{V_{max}/K_m + P_{dif}} \times 100 \quad (4)$$

The analysis of uptake study using OATP1B1- and OATP1B3-expressing HEK293 cells was carried out as follows. The uptake volume (µl/mg protein) was calculated by dividing the amount of uptake (dpm/mg protein) by its concentration in the incubation mixture (dpm/µl). The uptake volume in control cells and OATP1B1- or OATP1B3-expressing HEK293 cells at the set time points are shown in Table 1.

The analysis of inhibition study examining E-sul, CCK-8 and nateglinide saturable hepatic uptakes using cryopreserved human hepatocytes was carried out as follows. The uptake volume V_d (µl/10^6 cells) was calculated as the amount of uptake (dpm/10^6 cells) divided by its concentration in the incubation mixture (dpm/µl). The hepatic uptake clearance ($CL_{(2\ min\text{-}0.5\ min)}$) was calculated by the slope of the uptake volume V_d (µl/10^6 cells) between 0.5 and 2 min (equation 5). The saturable component of the hepatic uptake clearance (CL_{hep}) was determined by subtracting $CL_{(2\ min\text{-}0.5\ min)}$ in the presence of excess substrate from that in the presence of a tracer concentration of the substrate (equation 6).[22] The respective concentrations of E-sul, CCK-8 and nateglinide were set as follows, tracer: 0.005, 0.02 and 5 µmol/l, excess: 100, 100 and 1000 µmol/l.

Table 1 Time profiles for the uptake of [^{14}C]nateglinide in OATP1B1- and OATP1B3-expressing HEK293 and vector-transfected control cells

		Incubation time (min)			
		0.5	2	5	10
Uptake volume (µl/mg protein)	OATP1B1-expressing HEK293 cells	9.30 ± 0.69	24.0 ± 1.7	38.2 ± 0.9	42.7 ± 0.6
	Control cells of OATP1B1	9.43 ± 0.57	22.8 ± 0.6	31.7 ± 0.8	31.4 ± 0.6
	OATP1B3-expressing HEK293 cells	10.9 ± 0.7	18.9 ± 0.4	23.4 ± 1.3	26.8 ± 0.6
	Control cells of OATP1B3	9.11 ± 0.49	15.2 ± 1.2	20.2 ± 0.5	21.9 ± 1.3

Values are the average ± SD (n = 3) of actual measurements. [^{14}C]nateglinide concentration was equivalent to 10 µmol/l (OATP1B1) and 1 µmol/l (OATP1B3).

$$CL_{(2min-0.5min)} = \frac{V_{d,2min} - V_{d,0.5min}}{2 - 0.5} \quad (5)$$

$$CL_{hep} = CL_{(2min-0.5min),tracer} - CL_{(2min-0.5min),excess} \quad (6)$$

The ratio of CL_{hep} generated in the presence of each inhibitor to that in its absence was calculated as residual activity (%) (equation 7).

$$Residual\ activity\ (\%) = \frac{CL_{hep,+inhibitor}}{CL_{hep,no\ inhibitor}} \times 100 \quad (7)$$

The drug–drug interaction was analysed as follows. The 50% inhibitory concentration (IC50) was calculated from the obtained inhibition curves using WinNonlin Professional (Pharsight Corp., Mountain View, CA, USA). It was estimated from the increasing rate of the *AUC* of substrates after concomitant administration of two drugs based on the 1 + [*I*]/IC50 (where [*I*] = the concentration of the inhibitor adjacent to the transporter). Since it is impossible to know the exact value of [*I*] in humans, the maximum conceivable concentration at the time the inhibitor reaches the liver, $I_{in,max}$ was calculated from equation 8:[5,25]

$$I_{in,max} = I_{max} + \frac{k_a \times D \times F_a}{Q_h} \quad (8)$$

Where k_a is the absorption rate constant, D is dosage, F_a is the fraction absorbed from the gut into the portal vein, Q_h is hepatic blood flow, and I_{max} is the maximum blood concentration of the inhibitor. This study examined drug–drug interactions based on the concentration of protein not bound to drugs, and so the maximum unbound concentration of the inhibitor ($I_{in,max,u}$) was calculated by multiplying $I_{in,max}$ by the blood unbound fraction (f_u), and the value obtained was used as an approximation of [*I*].

Each parameter was collected from the data described in the references and the calculated F_a was assumed to be 1 to avoid a false negative prediction. K_a was calculated from equations 9 and 10 after determining the time to reach the maximum concentration (T_{max}) and the half-life ($t^1/_2$) from the references.

$$k_{el} = \frac{\ln 2}{t^{1}/_{2}} \quad (9)$$

$$T_{max} = \frac{\ln(k_a/k_{el})}{k_a - k_{el}} \quad (10)$$

K_{el} is the elimination rate constant. The value of a blood-to-plasma concentration ratio (R_B) is needed to calculate the unbound fraction in blood (f_u). The f_u values was calculated using an R_B value of 0.575 to avoid false negative prediction on the assumption that the drugs do not transfer to blood cells (R_B value = 1 – haematocrit; the value of 0.425 was used to determine haematocrit based on human biochemical data). When values obtained from the references varied within some range (such as 1.5–2), the median value was to be used.

Results

The results of the concentration dependency of uptake velocity of nateglinide and that of the fitting by the nonlinear least-squares method with a solid line using cryopreserved human hepatocytes are shown in Figure 1. The kinetic parameters for the uptake of nateglinide were as follows, K_m 51.6 μmol/l, V_{max} 337 pmol/min/10^6 cells, P_{dif} 7.19 μl/min/10^6 cells. Uptake of nateglinide was divided into saturable uptake and nonsaturable uptake using parameters obtained by fitting; the results are shown in Figure 1 as a dotted line (nonsaturable uptake) and as a dashed line (saturable uptake). Figure 1 shows the uptake profile of nateglinide by cryopreserved human hepatocytes to be an almost straight line, and it did not clearly reach a peak. The contribution rate of saturable hepatic uptake, based on the analyses of fitting, was 47.6%. On the other hand, the contribution rate of saturable hepatic uptake was 86.6% when E-sul (positive control) was used as a substrate in this experimental system.

Time-profiles for the uptake of nateglinide in OATP1B1- and OATP1B3-expressing HEK293 and control cells are shown in Table 1. The examination was thought to be

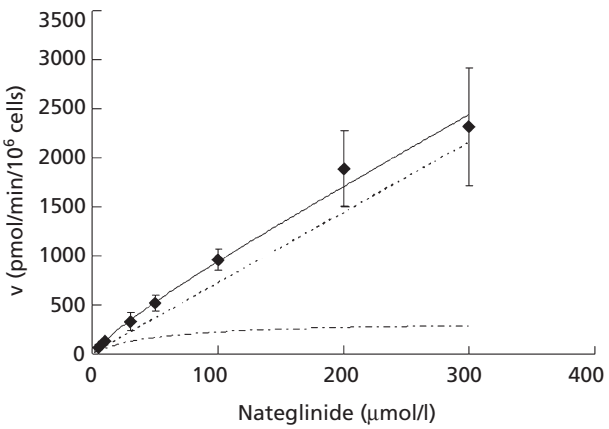

Figure 1 Concentration profiles for the uptake of [^{14}C]nateglinide by cryopreserved human hepatocytes. [^{14}C]nateglinide concentrations were 5, 10, 30, 50, 100, 200 and 300 μmol/l. The symbol represents the average ± SD (n = 3) of actual measurements. The solid line is a fitting curve obtained by the nonlinear least-squares method using MULTI. The dotted line is a nonsaturable uptake line, and the dashed line represents saturable uptake estimated from the kinetic parameter by fitting.

Table 2 Concentration dependency of the inhibitory effects of nateglinide and repaglinide on saturable uptake clearance of [^{3}H]estrone 3-sulfate and [^{3}H]cholecystokinin octapeptide by cryopreserved human hepatocytes

	[^{3}H]E-sul		[^{3}H]CCK-8	
Inhibitor	Inhibitor concentration (μmol/l)	Residual activity (%)	Inhibitor concentration (μmol/l)	Residual activity (%)
No inhibition	0	100	0	100
Nateglinide	3	95.6 ± 32.6	1	86.7 ± 18.8
	10	96.7 ± 35.5	3	77.3 ± 10.2
	30	80.7 ± 44.4	10	61.2 ± 12.8
	100	59.3 ± 26.7	30	38.8 ± 24.1
	300	39.8 ± 22.3	100	22.2 ± 13.2
Repaglinide	3	67.7 ± 20.0	0.3	91.7 ± 8.8
	10	48.4 ± 21.3	1	61.5 ± 13.2
	30	35.5 ± 10.2	3	55.6 ± 5.5
	100	19.7 ± 1.2	10	38.8 ± 15.5
	300	8.31 ± 2.43	30	26.8 ± 22.0
Rifampicin	300	25.8 ± 5.2	300	14.9 ± 25.9

Values are the average ± SD (n = 3) of actual measurements. E-sul, estrone 3-sulfate; CCK-8, cholecystokinin octapeptide.

appropriate because in the experiment conducted at the same time, the uptake of $E_2 17\beta G$ (positive control, uptake by OATP1B1-expressing cells was 0.05 μmol/l and by OATP1B3-expressing cells it was 1 μmol/l, the uptake time was 2 min in both experiments) by OATP1B1- and OATP1B3-expressing HEK293 cells was approximately 16- and 2.1-times higher than that by control cells, respectively, indicating an adequate uptake. Uptake of nateglinide in OATP1B1-expressing HEK293 cells clearly exceeded that in control HEK293 cells at 5 and 10 min, and the peak ratio was 1.4 at 10 min. Uptake of nateglinide in OATP1B3-expressing HEK293 cells exceeded the control cell value at all time points, and the ratio peaked at 10 min, 1.2-times.

The inhibitory effects of nateglinide and repaglinide on saturable uptake clearance of E-sul and CCK-8 in cryopreserved human hepatocytes are shown in Table 2. Nateglinide and repaglinide inhibited saturable uptake clearance of E-sul and CCK-8 in a concentration-dependent manner, and in both substrates the inhibitory effect was stronger with repaglinide than with nateglinide. Rifampicin was used as a positive control for inhibition of OATP1B1 and OATP1B3, and the examination was thought to be appropriate because an adequate inhibitory effect of rifampicin was obtained for both in the experiment (Table 2). The inhibitory effect of ciclosporin for saturable uptake clearance of nateglinide in cryopreserved human hepatocytes is shown in Figure 2. The inhibitory effects were weak up to 3 μmol/l ciclosporin, but an inhibitory effect exceeding 50% was obtained at a ciclosporin concentration of 10 μmol/l. The IC50 value obtained by the analysis using cryopreserved human hepatocytes and the $1 + I_{in,max,u}$/IC50 value obtained by this IC50 and calculation from clinical data are shown in Table 3.[3,26,27] The $1 + I_{in,max,u}$/IC50 value of nateglinide to saturable uptake of E-sul and CCK-8 were 1.008 and 1.080, respectively. The $1 + I_{in,max,u}$/IC50 value of ciclosporin to saturable uptake of nateglinide was 1.143 and all values were close to 1.

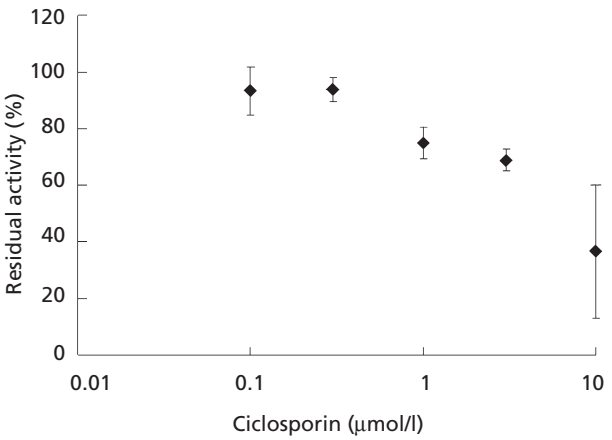

Figure 2 Concentration dependency of the inhibitory effect of ciclosporin on saturable uptake clearance of [^{14}C]nateglinide by cryopreserved human hepatocytes. The symbol represents the average ± SD (n = 3) of actual measurements.

Discussion

We have investigated the contributions of uptake transporters in the liver, OATP1B1 and OATP1B3, for nateglinide, and the possibility of drug–drug interactions via these transporters using transporter-expressing HEK293 cells and cryopreserved human hepatocytes.

The uptake profile of nateglinide into cryopreserved human hepatocytes was an almost straight line, and the

Table 3 IC50 values and $1 + I_{in,max,u}$/IC50 values for the inhibition study using cryopreserved human hepatocytes

Substrate Inhibitor	E-sul nateglinide	CCK-8 nateglinide	Nateglinide ciclosporin
Inhibitor parameter			
Dose (mg)		120[26]	344[27]
$t^1/_2$ (h)		1.8[26]	10.7[27]
T_{max} (h)		0.5[26]	1.5–2[27]
C_{max} (μg/ml)		8.5[26]	1.333[27]
Protein binding (%)		98[3]	93[27]
IC50 (μmol/l)	168 (100, 236)	17.4 (13.4, 21.3)	6.05 (1.82, 10.3)
$1 + I_{in,max,u}$/IC50	1.008	1.0 80	1.143

Values in parentheses are 95% confidence intervals (lower, upper). WinNonlin professional was used to calculate the 50% inhibitory concentration (IC50) values. E-sul, estrone 3-sulfate; CCK-8, cholecystokinin octapeptide; C_{max}, maximum concentration; $t^1/_2$, half-life; T_{max}, time to reach maximum concentration.

contribution rate of saturable hepatic uptake from the analyses of fitting was 47.6%. The contribution of the saturable hepatic uptake ratio of compounds such as cerivastatin and E-sul, known to be transporter substrates, was large in many cases.[17,22,23] Actually, the contribution rate of saturable hepatic uptake of E-sul in this experimental system was 86.6%. However, the contribution rate of the nonsaturable hepatic uptake exceeded that of the saturable hepatic uptake ratio for nateglinide. This suggested that hepatic uptake of nateglinide might have involved the active transporter, though the contribution of passive diffusion was larger.

The possibility of nateglinide being a substrate for OATP1B1 and OATP1B3 was examined in the uptake study using OATP1B1- and OATP1B3-expressing HEK293 cells. It was thereby shown that nateglinide could be a substrate for these transporters, because substantial uptake by expressing cells was observed as compared with the control cells for both transporters. In the uptake of nateglinide, the ratio of transporter-expressing HEK293 cells to control cells was less than 1.5 for each transporter, and this was considered to support the results obtained with cryopreserved human hepatocytes, assuming the contribution of passive diffusion to be large.

Repaglinide has often been reported to be a substrate of OATP1B1, and a clinical impact was suggested.[6,7,28] However, it was reported for nateglinide that *SLCO1B1* (encoding OATP1B1) polymorphism had no significant effect on the pharmacokinetics or pharmacodynamics of nateglinide or its M7 metabolite.[7] Furthermore, the plasma concentrations of repaglinide were reported to be significantly lower in individuals with the *SLCO1B1*1B/1B* genotype than in those with the *SLCO1B1*1A/*1A* genotype, but the *SLCO1B1*1B/1B* genotype slightly shortened the T_{max} of nateglinide while having no other effects on its pharmacokinetics.[29] On the other hand, there is a report suggesting that OATP1B1-mediated hepatic uptake of nateglinide may be a step before its metabolism and elimination.[30] Though findings on the contribution of OATP1B1 to nateglinide are very vague, the results of this study may support the above reports indicating that nateglinide could be recognized by OATP1B1 and OATP1B3, largely because of passive diffusion. The contribution of active transport to overall hepatocytic uptake is less significant. Therefore, the possibility of any clinical impact is considered to be low.

Cryopreserved human hepatocytes were used to examine the drug–drug interactions of nateglinide via OATP1B1 and OATP1B3. E-sul and CCK-8 are reportedly selective substrates of OATP1B1 and OATP1B3, respectively.[31,32] The inhibitory effect of nateglinide on OATP1B1 and OATP1B3 was estimated by obtaining the inhibition constant of nateglinide to the saturable uptake clearance of these compounds using cryopreserved human hepatocytes. To estimate the increase ratio of *AUC*, $1 + I_{in,max,u}$/IC50 was calculated from the IC50 and clinical data, and was close to 1 with each transporter. This $1 + I_{in,max,u}$/IC50 method avoids false negative predictions, and has been used widely as a prediction technique for drug–drug interactions via CYP. Furthermore, this method has been used for the prediction of drug–drug interactions via transporters.[18] The fact that the $1 + I_{in,max,u}$/IC50 value was close to 1 indicated that, in the case of combination administration of substrates for OATP1B1 or OATP1B3 and nateglinide, the possibility of increased *AUC* of the substrates via inhibitory effects of nateglinide was extremely low. The International Transporter Consortium described how to study transporters in drug development.[33] They stated that, to assess the potential of a compound to inhibit OATPs, the uptake of a prototypical substrate in a heterologous expression system for OATP1B1 or OATP1B3 should be measured in the presence of the compound, and the IC50 value should be determined. Furthermore, if the IC50 value is < 10-times the unbound C_{max}, then the compound may be an in-vivo OATP inhibitor. If this criterion is met, it is recommended that an in-vitro–in-vivo extrapolation approach ($1 + I_{in,max,u}$/IC50) be considered. If the $1 + I_{in,max,u}$/IC50 is higher than 2, a clinical drug–drug interaction study should be performed. The unbound C_{max} of nateglinide was calculated to be 0.54 μmol/l and the tenfold value was thus 5.4 μmol/l.[3,26] The IC50 values to OATP1B1 and OATP1B3 of nateglinide both exceeded this value. Therefore, the possibility of nateglinide inhibiting OATP1B1 and OATP1B3 was judged to be low from the viewpoint of comparison of the IC50 with the tenfold value of unbound C_{max}.

To examine the possibility of drug–drug interactions of nateglinide via inhibition of these active uptake transporters, the IC50 of ciclosporin to saturable uptake clearance of nateglinide was measured using cryopreserved human

hepatocytes. Ciclosporin is reportedly an inhibitor of many transporters containing OATP1B1, OATP1B3, OATP2B1 and P-glycoprotein.[33] To estimate the possibility of drug–drug interactions, $1 + I_{in,max,u}$/IC50 was calculated from the IC50 and clinical data, and was found to be close to 1. In addition, considering that the contribution of passive diffusion of nateglinide was large in the hepatic uptake shown in the aforementioned results, nateglinide may have a low possibility of drug–drug interactions based on ciclosporin inhibiting active uptake transporters.

Conclusions

It was suggested that nateglinide may serve as a substrate for OATP1B1 and OATP1B3, but the possibility of causing drug–drug interactions via these transporters was low.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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