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Research Paper

Inhibition of human liver microsomal CYP by nateglinide

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Abstract

Objectives Nateglinide is metabolized by CYP2C9 and CYP3A4, therefore drug–drug interactions through cytochrome P450 (CYP) inhibition may occur. In this study, we examined the inhibitory effects of nateglinide and its major metabolite *N*-[*trans*-4-(1-hydroxy-1-methylethyl)-cyclohexanecarbonyl]-D-phenylalanine (M1) on various CYP isoforms in human liver microsomes.

Methods We used typical substrates (7-ethoxyresorufin for CYP1A1/2, tolbutamide for CYP2C9, S-mephenytoin for CYP2C19, bufuralol for CYP2D6, chlorzoxazone for CYP2E1 and midazolam for CYP3A4) in the evaluation of the inhibitory effects, and examined the possibility of mechanism-based inhibition (MBI) by evaluating the influence of pre-incubation in the inhibition.

Key findings The results showed that nateglinide inhibited CYP2C9 and CYP2C19 with an $IC50_{app}$ (apparent value of the 50% inhibitory concentration) of 125 μmol/l and 946 μmol/l, respectively, while M1 did not inhibit any of the CYP isoforms. The inhibition constant (K_i) value of the inhibitory effect of nateglinide on CYP2C9 and the $1 + I_{in,max,u}/K_i$ value were estimated (where $I_{in,max,u}$ = the maximum unbound concentration of nateglinide). The $1 + I_{in,max,u}/K_i$ value was 1.02 (close to 1), suggesting a low risk of drug–drug interactions. The influence of pre-incubation on the inhibition by nateglinide of CYP3A4, CYP2C9 and CYP2C19 was examined. The results revealed that the inhibition of CYP by nateglinide was not influenced by pre-incubation, and that the possibility of MBI is very low.

Conclusions The possibility of drug–drug interactions involving nateglinide that might be attributable to CYP inhibition is low.

Keywords CYP; drug–drug interaction; nateglinide; prediction

Introduction

Nateglinide is widely used as an antidiabetic drug in patients with type 2 diabetes, and promotes insulin secretion and reduces hyperglycaemia after meals. The drug is known to be metabolized in the liver, mainly by CYP2C9 and partially by CYP3A4.[1] The major metabolite of nateglinide in humans is *N*-[*trans*-4-(1-hydroxy-1-methylethyl)-cyclohexanecarbonyl]-D-phenylalanine (M1); in addition, at least eight other metabolites have been detected in human plasma and urine.[2]

As shown for terfenadine and ketoconazole, drug–drug interactions may be associated with significant adverse reactions.[3] Cytochrome P450 (CYP) has been shown to be frequently involved in clinically problematic drug–drug interactions, and the potential for such interactions should be carefully considered when CYP is involved in the metabolism of drugs. Oral antidiabetic drugs, including nateglinide, are often administered for prolonged periods of time and unexpected drug interactions may occur depending on the concomitantly administered drug, as shown for the cases of gemfibrozil and repaglinide.[4] Thus, it is important to accumulate information regarding drug–drug interactions when clinical use of a drug is considered. We have already reported the potential for drug interactions of nateglinide with other drugs through inhibition of CYP enzymes involved in the metabolism of this drug.[5] In that study, we determined the possibility of interaction of nateglinide with 18 other drugs (metformin, buformin, aspirin, gemfibrozil, simvastatin, pioglitazone, rosiglitazone, carbamazepine, clarithromycin, gliclazide, clofibrate, fluconazole, bezafibrate, phenylbutazone, nifedipine, famotidine, ibuprofen and miconazole) by examining, using liver microsomes, the inhibitory effects of these 18 drugs on the conversion of

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nateglinide into its major metabolite M1. The results showed that the frequency of interactions of nateglinide with the aforementioned drugs is low, except for miconazole and fluconazole, which are potent inhibitors of CYP isoforms.[5] On the other hand, no detailed reports are available concerning the interactions of nateglinide with CYP inhibitors. This study was thus designed to predict the interaction of nateglinide with other drugs by examining its inhibition of various CYP isoforms in human liver microsomes. The following six major isoforms of CYP were examined: CYP1A1/2, CYP2C19, CYP2C9, CYP2D6, CYP2E1 and CYP3A4.

Materials and Methods

Chemicals and reagents

The test compounds, nateglinide and M1, and internal standard used when assaying nateglinide, *N*-(*trans*-4-*t*-butylcyclohexylcarbonyl)-D-phenylalanine, were synthesized by Ajinomoto Co., Inc. (Tokyo, Japan). Other chemicals used for the study were obtained from the following sources: *p*-acetophenetidine, imipramine hydrochloride, tolbutamide, quinidine, sodium *N*, *N*-diethyldithiocarbamate trihydrate, midazolam, ketoconazole and mefenamic acid from Wako Pure Chemical Industries, Ltd (Osaka, Japan); 7-ethoxyresorufin, α-naphthoflavone, dicoumarol, tranylcypromine, sulfaphenazole, 6-hydroxychlorzoxazone, chlorpropamide and chlorzoxazone from Sigma Chemical Co. (St Louis, USA); 4′-hydroxymephenytoin, S-mephenytoin, hydroxytolbutamide, bufuralol and (±)-hydroxybufuralol from Sumika Chemical Analysis Service, Ltd (Osaka, Japan); resorufin sodium salt and 1′-hydroxymidazolam from UFC, Ltd (Manchester, UK). Pooled human liver microsomes from 50 individuals were obtained from Xeno Tech, LLC (Kansas City, USA). β-Nicotinamide-adenine dinucleotide phosphate-reduced form (β-NADPH), D-glucose 6-phosphate, disodium salt (G-6-P) and glucose-6-phosphate dehydrogenase from yeast (G-6-P DH) were obtained from Oriental Yeast Co., Ltd (Tokyo, Japan). All the solvents and other chemicals used were of HPLC grade or of the highest purity available commercially.

In-vitro study

The volume of the reaction mixture containing the human liver microsomes was 0.5 ml, and the composition was 0.1 mmol/l EDTA and 0.1 mol/l potassium phosphate buffer (buffer, pH 7.4). The isoforms evaluated and the corresponding standard substrates, as well as the metabolites determined, the incubation time and the concentration of the human liver microsomal protein are shown in Table 1. These reaction conditions were used, based on a preliminary study, so as to generate metabolites in a linear manner depending on the time and protein concentration. A solution prepared by mixing the buffer, microsomes, test substance and substrate was equilibrated at 37°C for 5 min, and the reaction was started by the addition of the NADPH generating system (the final solution containing β-NADPH 2 mmol/l, G-6-P 19 mmol/l, magnesium chloride hexahydrate 10 mmol/l and G-6-P DH 2 U). Instead of the test substance, solvent was added to samples that did not contain an inhibitor. The test substance was not used in the investigation of $K_{m,app}$ and V_{max} values. Dicoumarol 1 mmol/l was also mixed in the reaction mixture to evaluate CYP1A1/2. The K_m values calculated in the evaluation system were used to express the final concentration of each substrate in the evaluation of the inhibitory effects (Table 1). Three concentrations (i.e. $\frac{1}{2} \times K_m$, K_m, and $2 \times K_m$) were used for calculating the $K_{i,app}$ values. The final concentration of the organic solvent used to dissolve the evaluated compound and each substrate was set at 1% or less. The reaction was terminated by the addition of a 3-fold volume of an organic solvent containing the internal standard to the reaction mixture. The internal standard used is shown in Analysis of compounds. As the organic solvent, methanol was used for resorufin and hydroxybufuralol, and acetonitrile was used for other compounds. No internal standard was used for hydroxytolbutamide. The solution was centrifuged at 15 000*g* for 10 min, and the supernatant was analysed. The sample of 6-hydroxychlorzoxazone was diluted 4-fold with 5 mmol/l ammonium acetate aqueous solution. The sample of resorufin was diluted 2.5-fold with water. The sample of hydroxybufuralol was diluted 3-fold with 75% methanol and determined.

Influence of pre-incubation on the inhibitory effects of nateglinide on the CYP isoforms

The experiment was performed using the same procedure as described in the previous section. However, the typical substrates were not added, and the buffer, microsome and test substance (nateglinide) were equilibrated at 37°C for 5 min. Pre-incubation was started by the addition of the NADPH generating system, and the reaction was started by the addition of the substrate after 30 min pre-incubation. The subsequent operations followed the same procedure described above.

Determination of the unbound fraction of nateglinide in the microsome reaction system

The fraction of nateglinide unbound to human liver microsomes ($f_{u,in\ vitro}$) was determined by the ultracentrifugation method by referring to the HAB protocol.[6] Buffer, microsome (final concentration 1.2 mg/ml), nateglinide (concentration when the $K_{i,app}$ value was calculated) and water were added and stirred for adjustment of the final volume to 0.5 ml. After incubation at 37°C for 5 min, it was centrifuged at 105 000*g* for 60 min. The concentration of nateglinide obtained in the supernatant was quantified. That value was set as $f_{u,in\ vitro}$ by dividing it by the concentration of nateglinide in the sample prepared using buffer, nateglinide and water only.

Analysis of compounds

The concentrations of the metabolites of the typical substrates in the reaction mixture were quantified by LC-MS/MS. The LC system used for resorufin was Shimadzu prominence series (Shimadzu Corporation, Kyoto, Japan), while that used for the other compounds was Agilent 1100 HPLC Series (Agilent Technologies, Wilmington, DE, USA). The MS/MS system used for resorufin was the API4000 system (Applied Biosystems, Foster City, USA), while that used for the other compounds was API3000 system (Applied Biosystems). The

Table 1 Substrates, metabolites, conditions of the reaction, basic kinetic parameters and inhibition parameters of the selected inhibitors used for this evaluation system

Isoform	CYP1A1/2	CYP2C19	CYP2C9	CYP2D6	CYP2E1	CYP3A4
Substrate	7-Ethoxyresorufin	S-Mephenytoin	Tolbutamide	Bufuralol	Chlorzoxazone	Midazolam
Incubation time (min)	10	30	30	12	10	3
Protein concn (mg/ml)	0.4	0.8	1.2	0.4	0.2	0.2
$K_{m,app}$ (μmol/l)	0.448	54.9	354	12.4	189	4.88
	(0.402, 0.494)	(46.3, 63.4)	(312, 396)	(10.2, 14.6)	(159, 219)	(2.93, 6.82)
V_{max} (pmol/min/mg protein)	21.0	86.4	216	108	3620	2130
	(19.9, 22.0)	(79.7, 93.1)	(200, 232)	(96.7, 118)	(3340, 3900)	(1570, 2700)
Selected inhibitor	α-Naphthoflavone	Tranylcypromine	Sulfaphenazole	Quinidine	Diethyldithiocarbamate	Ketoconazole
$IC50_{app}$ (μmol/l)	0.0162	17.7	2.16	0.0782	26.6	0.0233
	(0.0135, 0.0190)	(11.1, 24.3)	(0.696, 3.63)	(0.0383, 0.118)	(11.4, 41.8)	(0.0103, 0.0362)
$K_{i,app}$ (μmol/l)	0.00807	6.13	1.20	0.0522	28.9	0.0252
	(0.00150, 0.0146)	(3.17, 9.09)	(0.884, 1.52)	(0.0186, 0.0858)	(24.7, 33.1)	(0.0147, 0.0357)

The experiment was performed in triplicate using pooled human liver microsomes from 50 individuals. The reaction time for CYP2C19 was 60 min only for calculation of the $K_{m,app}$ and V_{max} values. For this reaction system, linearity up to 60 min was confirmed. The reaction time for CYP3A4 was 4 min only for calculation of the $K_{m,app}$ and V_{max} values. For this reaction system, linerarity upto 6 min was confirmed. For calculating the $IC50_{app}$, the K_m values were used to express the concentrations of the substrates. For calculating the $K_{i,app}$ values, three concentrations, (½ × the K_m value, the K_m value, and 2 × the K_m value) were used as the substrate concentrations. WinNonlin professional was used for the calculation of the $IC50_{app}$ value. Values in parentheses are the 95% confidence intervals (lower, upper).

columns used were Develosil ODS-UG-3 2 × 50 mm, 3 μm (Nomura Chemical Co., Ltd, Aichi, Japan). The columns were used at 55°C. The following mobile phases were used at a flow rate of 0.3 ml/min in a graded manner: 5 mmol/l ammonium acetate aqueous solution (mobile phase A) and acetonitrile (mobile phase B) for 6-hydroxychlorzoxazone and an aqueous solution of 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B) for other compounds. The gradient used was as follows: 6-hydroxychlorzoxazone: 2% mobile phase B from 0 min to 2 min and 4 min linear increase from 2% to 60% mobile phase B and 2% mobile phase B for 2 min; other compounds: 5 min linear increase from 5% to 60% mobile phase B and 60% mobile phase B for 1 min and 5% mobile phase B for 2 min. The MS/MS system, other parameters and internal standards are shown below in the order of Q1, Q3, collision (eV), polarity, internal standard used at evaluation of nateglinide and positive control, and internal standard used at evaluation of M1.

Resorufin: 214.0, 186.0, 37, positive, *p*-acetophenetidine (same as at evaluation of M1);

4′-Hydroxymephenytoin: 235.0, 150.0, 25, positive, imipramine, *p*-acetophenetidine;

Hydroxytolbutamide: 287.0, 89.1, 63, positive, no internal standard was used;

(±)-Hydroxybufuralol: 278.1, 186.0, 27, positive, imipramine, *p*-acetophenetidine;

6-Hydroxychlorzoxazone: 183.9, 120.0, –26, negative, chlorpropamide, mefenamic acid;

1′-Hydroxymidazolam: 342.0, 202.9, 37, positive, imipramine, *p*-acetophenetidine.

All analytes were detected using the multiple reaction monitoring mode.

The nateglinide was quantified by LC-MS/MS. *N*-(*trans*-4-*t*-butylcyclohexylcarbonyl)-D-phenylalanine was used as the internal standard. The LC system and the MS/MS system were the Agilent 1100 HPLC Series and API3000 system, respectively. The columns used were Develosil ODS-UG-3 2 × 50 mm, 3 μm. The columns were used at 55°C. The following mobile phases were used at a flow rate of 0.3 ml/min in a graded manner: an aqueous solution of 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B). The gradient used was as follows: 6 min linear increase from 10% to 70% mobile phase B and 70% mobile phase B for 3 min and 10% mobile phase B for 1 min. Q1, Q3, collision (eV), and polarity were 318.30, 166.20, 27 and positive, respectively.

Calculation of the parameters and statistical method

All experiments were performed in triplicate using pooled human liver microsomes from 50 individuals. The apparent values of the Michaelis constant ($K_{m,app}$) and maximum velocity (V_{max}) were calculated by the Eadie–Hofstee plot (as for the $K_{m,app}$ and V_{max}, the 95% confidence intervals were calculated based on the least-squares method using JMP 6.0 (SAS Institute, Cary, USA) by the Eadie–Hofstee plot). The apparent values of the 50% inhibitory concentration ($IC50_{app}$) and inhibition constant (K_i) were calculated basically by referring to the HAB protocol.[6] The ratio of the concentrations of the metabolites of the typical substrates generated in the presence of the test substances to those in the absence of the test substances was calculated as the residual activity (%) for the respective inhibitors. The $IC50_{app}$ values were calculated using WinNonlin Professional (Pharsight Corp., Mountain View, USA) by plotting the residual activity versus the concentration of the test substance. The 95% confidence intervals were calculated using the same analysis software. When the inhibition was < 50% at the maximum concentration, the $IC50_{app}$ value was defined as larger than the maximum concentration. The $K_{i,app}$ values for selective inhibitors were calculated from a Dixon plot (as for the $K_{i,app}$ values of selective inhibitors, the 95% confidence intervals were calculated based on the least-squares method using JMP 6.0

by the Dixon plot). As to CYP2C9, for which obvious inhibitory effects of nateglinide was observed, the K_i value was calculated from a Dixon plot, using the concentration of the unbound form obtained by multiplying the concentration added to the unbound fraction ($f_{u,in\ vitro}$) in the reaction system. Regarding the effects of pre-incubation, the inhibition curves in the presence and absence of pre-incubation were shown together and a significant difference between the two was evaluated by a test of slope homogeneity ($P < 0.05$) using SAS 9.1 (SAS Institute, Cary, USA).

Prediction of drug–drug interactions

We estimated the percent increase of the AUC (area under the curve) of the drug after concomitant administration of nateglinide, based on the $1 + I_{in,max,u} / K_i$ (where $I_{in,max,u}$ = the maximum unbound concentration of nateglinide) for the drug exhibiting potent inhibitory effects, which was calculated by a previously reported method.[5]

Results

The kinetic parameters for individual reactions calculated in the present system for evaluating CYP inhibition, and the $IC50_{app}$ value obtained in the inhibition study using a selected inhibitor for each reaction and the $K_{i,app}$ value calculated from the Dixon plot are shown in Table 1. In addition, the K_m and K_i values reported to date in the literature are summarized in Table 2.

The inhibitory effects of nateglinide and its major metabolite M1 on various P450 isoforms were evaluated by the assay system using the typical substrate for each isoform. Among the six isoforms evaluated for nateglinide, inhibition of four isoforms (CYP1A1/2, CYP2D6, CYP2E1 and CYP3A4) did not exceed 50% even at 1000 μmol/l, indicating that the $IC50_{app}$ was larger than 1000 μmol/l (Table 3). For CYP2C19 and CYP2C9, the decrease in enzyme activity was dependent on the concentration of nateglinide. The $IC50_{app}$ values obtained indicated potent inhibitory effects of the drug on CYP2C9 (Table 3), while M1 had no obvious inhibitory effects on any of the isoforms. Since the $IC50_{app}$ value of nateglinide for CYP2C9 was relatively low (125 μmol/l) and the inhibitory effect was high, the K_i value was calculated from the Dixon plot. The $f_{u,in\ vitro}$ was almost constant at 0.91, irrespective of the concentration added to the system. The K_i value obtained from the Dixon plot was 66.6 μmol/l (Table 4). The value of $(1 + I_{in,max,u} / K_i)$, which was calculated from the K_i value and the $I_{in,max,u}$ value estimated from the clinical data, was 1.02 (Table 4).

In addition, to explore the possibility of mechanism-based inhibition (MBI) by nateglinide, we examined the influence of pre-incubation on the inhibition of CYP2C19, CYP2C9 and CYP3A4 by nateglinide. CYP2C19 and CYP2C9 were selected as the targets for this evaluation, because the $IC50_{app}$ values were calculated from the inhibition experiment system. CYP3A4 was selected as the target, because in the event of occurrence of MBI, it is highly likely to cause serious adverse reactions, and a residual activity of this enzyme of 80% or less at high concentrations was obtained in the inhibition experiments. The residual activity was 80% or more for CYP1A1/2, CYP2D6 and CYP2E1 even at the maximum concentration of nateglinide. For three isoforms (CYP2C9, CYP2C19 and CYP3A4), pre-incubation with NADPH for 30 min failed to enhance its inhibition (Figure 1–3). As for the three isoforms, there was no statistically significant difference between the presence and absence of pre-incubation.

Discussion

The typical substrates and selective inhibitors of CYP used in this study are compounds commonly used for evaluation of the activity and inhibition of CYP. In this study, the basic kinetic parameters ($K_{m,app}$ and V_{max}) and the inhibition parameters ($IC50_{app}$ and $K_{i,app}$) for selective inhibitors were calculated from the experiments, and compared with the reported values to confirm the validity of the evaluation system. The results showed that the value of the inhibition constant, $K_{i,app}$, for each typical inhibitor calculated using this evaluation system was comparable with the reported values, thereby confirming that the evaluation system used was appropriate.

The in-vitro inhibitory effects of nateglinide on CYP, as well as the possibility of MBI, have not yet been elucidated in detail. This is the first study of inhibition of CYP isoforms by nateglinide. In this study, the inhibitory effects of nateglinide and M1 on six major CYP isoforms (CYP1A1/2, CYP2C19, CYP2C9, CYP2D6, CYP2E1 and CYP3A4) were evaluated, and high $IC50_{app}$ values were obtained for all of the isoforms, except for CYP2C9. Since these values were considerably higher than the C_{max} after clinical dosing with 120 mg (26.8 μmol/l)[19] of nateglinide, the possibility of drug–drug interactions associated with either competitive or non-competitive inhibition of these isoforms was determined to be low. To determine the inhibitory effects of nateglinide on CYP2C9, we carefully predicted the possibility of drug–drug interactions based on the value of $(1 + I_{in,max,u} / K_i)$. The value of $(1 + I_{in,max,u} / K_i)$ for CYP2C9 was 1.02

Table 2 Reported values of each parameter

Isoform	Substrate	Inhibitor	K_m (μmol/l)	V_{max} (pmol/min/mg protein)	K_i (μmol/l)
CYP1A1/2	7-Ethoxyresorufin	α-Naphthoflavone	0.20,[7] 0.519[8]	15.0[8]	0.010[7]
CYP2C19	S-Mephenytoin	Tranylcypromine	86.0,[8] 39,[9] 52.7,[11] 68.3[13]	8.54,[8] 102,[9] 100[13]	8.7[14]
CYP2C9	Tolbutamide	Sulfaphenazole	200,[7] 442,[8] 65,[9] 190,[10] 206[11]	84.9,[8] 194,[9] 500[10]	0.3,[7] 0.7[12]
CYP2D6	Bufuralol	Quinidine	21.7[8]	61.0[8]	0.03[15]
CYP2E1	Chlorzoxazone	Diethyldithiocarbamate	115,[8] 27,[9] 59.2,[10] 56.8,[11] 40[16]	2600,[8] 807,[9] 1930[10]	21[16]
CYP3A4	Midazolam	Ketoconazole	1.4,[9] 1.8,[13] 3.6[18]	1858,[9] 1300,[13] 3183[18]	0.059[17]

Table 3 $IC50_{app}$ values of nateglinide and M1 for each CYP isoform

	$IC50_{app}$ (μmol/l)	
	Nateglinide	M1
CYP1A1/2	> 1000	> 1000
CYP2C19	946 (792, 1100)	> 1000
CYP2C9	125 (93.9, 157)	> 1000
CYP2D6	> 1000	> 1000
CYP2E1	> 1000	> 1000
CYP3A4	> 1000	> 1000

The K_m value of each typical substrate was used as the concentration of the substrates. The experiment was performed in triplicate using pooled human liver microsomes from 50 individuals. WinNonlin professional was used for calculation of the $IC50_{app}$ value. Values in parentheses are the 95% confidence intervals (lower, upper).

Table 4 $1+I_{in,max,u}/K_i$ value of nateglinide for CYP2C9

Nateglinide parameter		Reference
Dose (mg)	120	Sabia *et al.*[19]
t½ (h)	1.8	Sabia *et al.*[19]
T_{max} (h)	0.5	Sabia *et al.*[19]
C_{max} (μg/ml)	8.5	Sabia *et al.*[19]
Protein binding (%)	98	McLeod *et al.*[1]
K_i (μmol/l)	66.6	Obtained value
$I_{in,max,u}$ (μmol/l)	1.38	Calculated
$1 + I_{in,max,u}/K_i$	1.02	Calculated

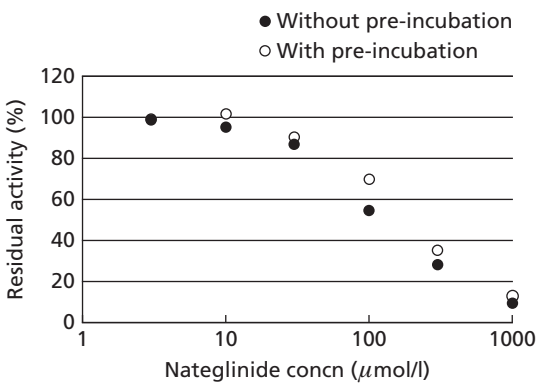

Figure 1 Inhibition curve of nateglinide against CYP2C9 and the influence of pre-incubation. The experiment was performed in triplicate using the K_m value of tolbutamide and pooled human liver microsomes from 50 individuals

(i.e. close to 1). Thus, it is highly unlikely for drug interactions involving nateglinide to occur under competitive or non-competitive inhibition of CYP2C9.

Unlike competitive or non-competitive inhibition, MBI occurs when the metabolite formed by an enzyme binds irreversibly to the enzyme, thereby inducing inhibition. This can result in serious adverse drug reactions. Compounds acting as mechanism-based inhibitors are characterized by the finding that in the in-vitro system used for evaluating inhibition, pre-incubation with NADPH enhances their inhibitory effects in a

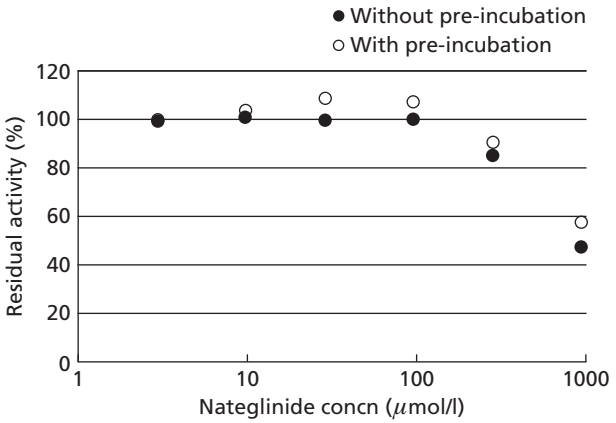

Figure 2 Inhibition curve of nateglinide against CYP2C19 and the influence of pre-incubation. The experiment was performed in triplicate using the K_m value of S-mephenytoin and pooled human liver microsomes from 50 individuals

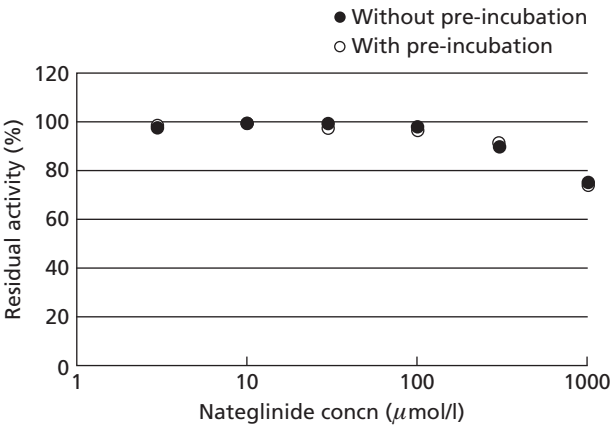

Figure 3 Inhibition curve of nateglinide against CYP3A4 and the influence of pre-incubation. The experiment was performed in triplicate using the K_m value of midazolam and pooled human liver microsomes from 50 individuals

manner dependent on the concentration and the pre-incubation time. Therefore, when drugs act as mechanism-based inhibitors, their inhibitory curve would shift toward the side of the lower concentration after pre-incubation. Pre-incubation did not enhance the inhibition of any of the three isoforms evaluated in this study, suggesting that nateglinide does not function as a mechanism-based inhibitor.

It has been reported that when nateglinide is used concomitantly with diclofenac (a substrate for CYP2C9), it does not affect the pharmacokinetics of diclofenac,[20] and another report has stated that concomitant use of nateglinide with warfarin (a substrate for CYP3A4 and CYP2C9) does not affect the pharmacokinetics in either direction.[21] These findings are supported by the results obtained in this in-vitro study.

Conclusions

The inhibitory effects of nateglinide and M1 on CYP were small. Even for CYP2C9, against which the drug exhibited the most

potent inhibition, the possibility of interactions is considered to be minimal, based on the value of $(1 + I_{in,max,u} / K_i)$. Moreover, considering that the possibility of MBI is very low, potential drug–drug interactions involving inhibition of CYP by nateglinide are considered to be minimal.

Declarations

Conflict of interest

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

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