

# Effect of the CYP2C8 Genotype on the Pharmacokinetics and Pharmacodynamics of Repaglinide

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## ABSTRACT:

The pharmacokinetics of repaglinide shows pronounced interindividual variability, for which several reasons have been considered, including interactions with drugs inhibiting CYP2C8 and CYP2C8 genetic polymorphism. However, existing data on the role of genetic polymorphisms in repaglinide disposition are not fully consistent. We studied the effect of CYP2C8\*3 on the pharmacokinetics and pharmacodynamics of repaglinide in 29 healthy whites carrying CYP2C8\*3/\*3 ( $n = 4$ ), CYP2C8\*1/\*3 ( $n = 13$ ), or CYP2C8\*1/\*1 ( $n = 12$ ). After administration of a single dose of 2 mg of repaglinide, blood was drawn for assessment of repaglinide pharmacokinetics and pharmacodynamics, and urine was collected to quantify the main repaglinide metabolites M1 and M4 up to 24 h postdose. Repaglinide and the metabolites were quantified by liquid chromatography-tandem mass spectrometry. Considering only the effect of CYP2C8\*3, the mean (95% confidence interval) area under the time-concentration curve

(AUC) from zero to infinity of repaglinide was 72.4 (6.7–138.0), 97.2 (59.2–135.2), and 105.9 (52.4–159.3)  $\text{ng} \cdot \text{ml}^{-1} \cdot \text{h}$  and the maximal concentration ( $C_{\text{max}}$ ) was 38.5 (3.8–73.2), 50.3 (37.5–63.0), and 60.3 (31.5–89.1)  $\text{ng} \cdot \text{ml}^{-1}$ , respectively, in carriers of CYP2C8\*3/\*3, CYP2C8\*1/\*3, and CYP2C8\*1/\*1 [ $p > 0.05$ , one-way analysis of variance (ANOVA)]. In addition, for urinary metabolite excretion and pharmacodynamic parameters, i.e., mean and maximal changes in insulin and glucose concentration, no significant differences between CYP2C8 genotypes were observed. Likewise, no significant effects on the pharmacokinetics or pharmacodynamics were observed when AUC and  $C_{\text{max}}$  of repaglinide were corrected for reported effects of the *SLCO1B1* 521T>C polymorphism or when both polymorphisms were tested in a two-way ANOVA. In conclusion, CYP2C8\*3 does not seem to play an important role in the pharmacokinetics and pharmacodynamics of repaglinide given in a therapeutic dose.

## Introduction

Repaglinide, a meglitinide analog, is a fast-acting insulin secretagogue drug used to normalize postprandial hyperglycemia in patients with type 2 diabetes. After oral administration it is rapidly absorbed with absolute bioavailability of approximately 60% (Hatorp, 2002). Repaglinide is extensively biotransformed in the liver to inactive metabolites, which are predominantly excreted via the feces and to approximately 8% via urine (van Heiningen et al., 1999). In vitro, both CYP3A4 and CYP2C8 were shown to be the crucial enzymes responsible for repaglinide metabolism with formation of M1 and M4 as the quantitatively most important metabolites. At the same time, at therapeutically relevant concentrations, CYP3A4 was principally responsible for the formation of M1, whereas M4 was the major metabolite generated via CYP2C8 (Bidstrup et al., 2003; Kajosaari et al., 2005a). The roles of CYP3A4 and CYP2C8 in repaglinide metabolism were also confirmed in clinical studies. The potent CYP3A4

inhibitors, clarithromycin and itraconazole, increased the area under the plasma concentration-time curves (AUCs) of repaglinide by 40%, whereas the CYP2C8 inhibitors trimethoprim and gemfibrozil led to an increase in AUC of the drug by approximately 60 and 700%, respectively (Niemi et al., 2001, 2003a, 2004). Parallel to numerous studies evaluating the impact of possible drug-drug interactions on the pharmacokinetics and dynamics of repaglinide, the role of genetic factors in the large interindividual variability of the drug pharmacokinetics was also studied.

CYP2C8 is a genetically polymorphic enzyme. The CYP2C8\*3 allele, which involves two linked amino acid substitutions R139K and K399R, is quite common in whites, showing a frequency of approximately 14% (Dai et al., 2001; Bahadur et al., 2002; Weise et al., 2004). CYP2C8\*3 is in linkage disequilibrium with the genetic variant \*2 in another enzyme, i.e., CYP2C9 (Yasar et al., 2002). Because CYP2C9\*2 is associated with reduced enzymatic activity to most substrates and there is some overlap between substrate recognition for CYP2C8 and CYP2C9, this linkage may contribute to clinical relevance of the CYP2C8\*3 variant. The role of the CYP2C8\*3 polymorphism has been explored in studies with various commonly prescribed pharmaceuticals, such as rosiglitazone, ibuprofen, or paclitaxel (Kirchheiner et al., 2006; Blanco et al., 2008; Gréen et al., 2009).

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**ABBREVIATIONS:** AUC, area under the plasma concentration-time curve; ANOVA, analysis of variance.

However, the results are partly conflicting, and no well defined impact on CYP2C8 activity has been identified. Likewise, there is no consistent evidence for the role of *CYP2C8\*3* in the metabolism of repaglinide. Niemi et al. (2003b, 2005) found decreased (by 45–48%) AUC values of repaglinide in heterozygous carriers of the *CYP2C8\*3* variant allele, suggesting that it is related to a higher metabolic activity of the enzyme. On the other hand, the study by Bidstrup et al. (2006) yielded no relevant differences in repaglinide pharmacokinetics with respect to this polymorphism.

At the same time, the effects of polymorphisms in *SLCO1B1*, the gene encoding organic anion-transporting polypeptide 1B1, which is responsible for the hepatic uptake of a broad range of endogenous substrates and drugs, have been considered in repaglinide pharmacokinetics and dynamics. One of the common *SLCO1B1* polymorphisms in whites is 521T>C (allelic frequency 14%), resulting in the amino acid substitution V174A and reduced transporter activity (Tirona et al., 2001). This polymorphism was a major factor influencing repaglinide pharmacokinetics, and significantly higher AUC values of the drug were observed in carriers of the 521CC variant compared with carriers of the 521TT genotype (Niemi et al., 2005; Kalliokoski et al., 2008a, b).

The aim of the present study was to evaluate the impact of the *CYP2C8\*3* polymorphism, with the inclusion of homozygous carriers of this variant, on the pharmacokinetics and pharmacodynamics of repaglinide when given in a clinically relevant dose of 2 mg and taking into account the main *SLCO1B1* polymorphism 521T>C.

### Materials and Methods

**Subjects.** Twenty-nine white subjects with various genotype constellations involving the *CYP2C8\*3* polymorphism were chosen from a large panel of pharmacogenetically characterized volunteers and asked to participate in the study. With regard to the previously published data on the pharmacokinetic variability of repaglinide (Hatorp, 2002), a sample size of at least three individuals with the homozygous *CYP2C8\*3* genotype was required to have a power of 80% at an  $\alpha$  of 5% to detect a difference considered as clinically relevant (100%) in the AUC value between the wild-type and homozygous *CYP2C8\*3* carriers. All subjects gave their written informed consent for the study and were healthy as confirmed on the basis of an extensive screening examination, none of them being a regular smoker.

**Study Design.** The Ethics Committee of the Faculty of Medicine of the University of Cologne reviewed and approved the study. A single dose of 2 mg of repaglinide (Novonorm coated tablet; Novo Nordisk, Bagsværd, Denmark) was administered together with 240 ml of water at approximately 7:00 AM after a 9-h fast. The subjects continued fasting for 6 h after taking the study medication. Furthermore, they were asked to abstain from alcohol, caffeine-containing beverages, and grapefruit products from 3 days before until 2 days after the dosing. Blood sampling for the determination of repaglinide concentration was performed 3 min before the dosing and at 15, 30, and 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h postdose (15 samples). At all time points, venous blood glucose was quantified using a photochemical method calibrated within the range of 1.1 to 33.3 mM (Accutrend sensor; Hestia, Mannheim, Germany); in addition, samples for insulin measurement were drawn up to 6 h postdose. Moreover, urine samples for quantification of repaglinide and its metabolites M1 and M4 were collected just before the dosing and in intervals from 0 to 4, 4 to 8, 8 to 12, 12 to 16, and 16 to 24 h. For safety evaluation, adverse events and well-being were surveyed by non-leading questions asked at each time of blood sampling.

**Analytical Methods.** Repaglinide and its metabolites M1 and M4 were determined using liquid chromatography-tandem mass spectrometry according to methods published previously (Ho et al., 2004; Kajosaari et al., 2005b). Repaglinide was measured in plasma and urine, whereas both metabolites were determined in urine only because of their undetectable concentrations in plasma.

Quantification of repaglinide was performed using peak area ratios of the analyte and the internal standard (diclofenac). Because reference compounds

for the metabolites were not available, M1 and M4 concentrations were given in arbitrary units relative to the peak area ratio of each metabolite to that of the internal standard in the chromatogram.

Linearity of the calibration curves was demonstrated for plasma samples between 0.098  $\mu\text{g/l}$  (lower limit of quantitation) and 79.139  $\mu\text{g/l}$  and for urine samples between 0.394  $\mu\text{g/l}$  (lower limit of quantitation) and 161.787  $\mu\text{g/l}$ . Precision ranged from 7.5 to 9.4% in plasma and from 4.8 to 10.7% in urine, whereas accuracy was 96.7 to 105.8% in plasma and 96.8 to 108.7% in urine.

Plasma insulin was measured using a solid-phase enzyme-labeled chemiluminescent immunometric assay (Insulin IMMULITE 2000; Siemens Healthcare Diagnostics Products Ltd., Llanberis, United Kingdom) with a calibration range of 2 to 300 mU/l.

**Genotyping Methods.** Genotyping for *CYP2C8\*3* was performed as described previously (Kirchheiner et al., 2006). All subjects were also genotyped for *CYP2C9* to control for *CYP2C9\*2* and *\*3* (Kirchheiner et al., 2004). Genotyping for *SLCO1B1* 521T>C was performed by means of allele-specific PCR, using the forward primers 5'-CATACATGTGGATATATGT-3' and 5'-CATACATGTGGATATATGC-3' for the wild-type and mutant variants, respectively (Tirona et al., 2001). The *SLCO1B1* 521T>C polymorphism was determined in subjects retrospectively to allow for the consideration of this genotype in the evaluation of the impact of *CYP2C8\*3* on repaglinide pharmacokinetics and dynamics.

**Pharmacokinetics/Pharmacodynamics.** Pharmacokinetic analysis was performed using WinNonlin (version 1.5; Pharsight, Mountain View, CA) in the noncompartmental mode. Maximal plasma concentrations of repaglinide were obtained directly from the measured values. The AUC of repaglinide was calculated using the combined linear and log-linear trapezoidal rule with extrapolation to infinity. The weight-adjusted oral clearances were calculated as dose divided by respective AUC and body weight. The amounts of repaglinide metabolites M1 and M4 excreted in urine up to 24 h postdose ( $A_{e,0-24h}$ ) were calculated by adding up the amounts eliminated during the respective urine sampling periods. For pharmacodynamic evaluation, maximal changes from the baseline glucose and insulin concentration as well as mean changes in glucose and insulin concentration, calculated as the average differences between the respective values measured up to 6 h postdose and the baseline values, were considered. All calculations were performed using Excel 2003 (Microsoft, Seattle, WA).

**Statistical Methods.** Mean values accompanied by 95% confidence intervals were calculated for all the pharmacokinetic and pharmacodynamic parameters of repaglinide in the overall study population and respective *CYP2C8\*3* genotype groups. To assess *CYP2C8*-mediated differences in pharmacokinetic and dynamic parameters, a one-way ANOVA with derived linear trend test on ANOVA was performed, whereas the cumulative effects of both genotypes *CYP2C8* and *SLCO1B1* 521T>C were evaluated using a two-way ANOVA. If the parameters appeared to be non-normally distributed, a log transformation was applied. Moreover, for both pharmacokinetic parameters, AUC and maximal concentration ( $C_{max}$ ), a one-way ANOVA was also performed on data corrected for the estimated effect of *SLCO1B1* 521T>C. To this end the AUC and  $C_{max}$  in carriers of *SLCO1B1* 521CT and CC were divided by a respective correction factor reflecting an assumed mean increase of these parameters due to the presence of one or two 521C alleles compared with the 521TT genotype. The correction factors were derived by means of a weighted linear regression on the basis of the previously published values of AUC and  $C_{max}$  observed in carriers of 521TT, CT, and CC, all noncarriers of the *CYP2C8\*3* allele, after the administration of a single dose of 0.25 to 2 mg of repaglinide (Niemi et al., 2005; Kalliokoski et al., 2008a,b). All statistical calculations were done by SPSS 14.0 (SPSS Inc., Chicago, IL).

### Results

Of 29 study participants, 15 were women and 14 were men. The mean  $\pm$  S.D. age was 35  $\pm$  12.7 years (range, 24–64 years), and the body mass index was 23.4  $\pm$  2.5 kg/m<sup>2</sup> (range, 18.6–29.3 kg/m<sup>2</sup>). Seventeen subjects were carriers of the *CYP2C8\*3* mutation (13 heterozygotes and 4 homozygotes) and 12 subjects were homozygous carriers of the *CYP2C8\*1* genotype (wild types). Moreover, in the population studied there was a complete linkage between *CYP2C8\*3* and *CYP2C9\*2*. With respect to the *SLCO1B1* 521T>C genotype, 18

TABLE 1

Pharmacokinetic parameters of repaglinide and urinary excretion of repaglinide metabolites M1 and M4 relating to the CYP2C8\*3 genotype

Data, obtained by noncompartmental analysis, are shown as mean (95% confidence interval).

Genotype/Parameter	AUC <sub>0-∞</sub>	C <sub>max</sub>	Oral Clearance/b.wt.	t <sub>1/2</sub>	T <sub>max</sub>	Ae <sub>0-24 h</sub>	
						M1	M4
	ng · ml <sup>-1</sup> · h	ng · ml <sup>-1</sup>	l · h <sup>-1</sup> · kg <sup>-1</sup>	h	h	AU	
All subjects (n = 29)	97.4 (71.1–123.7)	52.8 (40.1–65.4)	0.46 (0.34–0.57)	1.3 (1.0–1.5)	0.8 (0.7–1.0)	48.3 (30.3–66.3)	217.8 (172.0–263.6)
CYP2C8*1/*1 (n = 12)	105.9 (52.4–159.3)	60.3 (31.5–89.1)	0.45 (0.23–0.67)	1.2 (1.0–1.4)	0.8 (0.6–1.0)	52.8 (8.4–97.2)	184.2 (125.8–242.6)
CYP2C8*1/*3 (n = 13)	97.2 (59.2–135.2)	50.3 (37.5–63.0)	0.46 (0.28–0.64)	1.3 (0.8–1.8)	0.8 (0.6–1.0)	42.6 (28.2–57.1)	212.4 (152.4–271.9)
CYP2C8*3/*3 (n = 4)	72.4 (6.7–138.0)	38.5 (3.8–73.2)	0.48 (0.02–0.95)	1.3 (–0.5–3.1)	0.9 (0.5–1.2)	53.3 (21.5–85.0)	336.7 (11.6–661.7)
p, one-way ANOVA	0.717	0.505	0.979	0.891	0.900	0.641	0.457
p, one-way ANOVA trend test (linear contrast)	0.420	0.271	0.840	0.705	0.678	0.353	0.221

AUC<sub>0-∞</sub>, area under the plasma concentration time curve up to infinity; C<sub>max</sub>, maximal concentration; t<sub>1/2</sub>, terminal half-life; T<sub>max</sub>, time to reach maximal plasma concentration; Ae<sub>0-24 h</sub>, amount excreted in urine up to 24 h postdose; AU, arbitrary units.

subjects were homozygous wild types, 9 were heterozygous carriers, and 2 were homozygous carriers of the variant allele.

All participants completed the study. After administration of repaglinide up to the end of the fasting time (6 h postdose), blood glucose ranged from 2.1 to 8.2 mM. In 7 subjects, transient symptoms of hypoglycemia, such as dizziness, sweating, and tremor were observed but quickly disappeared after administration of 200 to 400 ml of apple juice. Otherwise, the study medication was well tolerated.

**Assessment of Effects of the CYP2C8\*3 Polymorphism.** *Pharmacokinetic results.* Table 1 shows the mean and 95% confidence interval for plasma and urine pharmacokinetic parameters of repaglinide in all study participants, stratified according to the CYP2C8 genotype. Although the mean values of AUC<sub>0-∞</sub> and C<sub>max</sub> decreased and, accordingly, the weight-adjusted oral clearance increased with the number of CYP2C8\*3 alleles, neither of the parameters differed significantly between the groups ( $p > 0.05$ , one-way ANOVA with derived linear trend test). The concentration-time profile of repaglinide up to 6 h postdose in carriers of the CYP2C8\*1/\*1, \*1/\*3, and \*3/\*3 genotypes is shown in Fig. 1.

Likewise, taking into account repaglinide metabolites excreted in urine up to 24 h, the mean amount of M4 but not of M1 and the ratio of M4 to AUC<sub>0-∞</sub> of repaglinide (data not shown) increased with the number of CYP2C8\*3 alleles. However, the observed trend was not statistically significant either.

*Pharmacodynamic results.* Considering the mean change in insulin concentration and the maximal observed change in insulin concentration up to 6 h postdose, the time when lunch was given, there was an apparent tendency toward a lower insulin increase in the groups with at least one variant allele, suggesting a genotype dose-dependent manner (Table 2; Fig. 2). As with the pharmacokinetic parameters, the observed differences in insulin secretion between the genotype groups did not reach the level of statistical significance ( $p > 0.05$ , one-way ANOVA with derived linear trend test). Moreover, the lower insulin secretion assessed in carriers of the variant allele was not reflected by corresponding changes in the glucose concentration (mean and maximal change in blood glucose) because the biggest glucose decrement was observed in carriers of the CYP2C8\*1/\*3 genotype (Table 2; Fig. 3). At the same time, four homozygous carriers of CYP2C8\*1 (33%) and three heterozygous carriers of CYP2C8\*3 (23%) were given apple juice (one carrier of CYP2C8\*1/\*3 needed a double portion of the juice) because of hypoglycemia-related adverse events. None of the four subjects with the CYP2C8\*3/\*3 genotype developed hypoglycemia.

**Assessment of Concomitant Effects of the CYP2C8\*3 and SLCO1B1 521T>C Polymorphisms.** To assess the potential concomitant effect of the SLCO1B1 521T>C polymorphism on repaglinide pharmacokinetics and dynamics, a two-way ANOVA with both

polymorphisms as factors was performed. Respective mean values and corresponding S.D. for resulting genotype groups are shown in Table 3. Because of the relatively small number of subjects treated in the study, none of the carriers of CYP2C8\*1/\*1 concurrently had the 521CC genotype, and in several genotype combination groups (CYP2C8\*1/\*3 and 521CC, CYP2C8\*3/\*3 and 521TT, and CYP2C8\*3/\*3 and 521CC) only one subject was identified in the population studied. Whereas for carriers of the 521TC genotype, a trend for decreasing values in AUC<sub>0-∞</sub>, C<sub>max</sub>, and the mean change in insulin and of increasing values in the amount of M4 excreted, with the number of the CYP2C8\*3 alleles was observed, no such trend was observed for 521TT and CC genotypes. However, except for a significant main effect of SLCO1B1 on the amount of M4 excreted in urine ( $p < 0.039$ ) and a significant interaction of the two genotypes with respect to this parameter ( $p < 0.026$ ), the two-way ANOVA revealed no statistical differences between the given genotype groups.

Irrespective of the two-way ANOVA test, we also assessed the potential effect of the CYP2C8\*3 genotype on the AUC<sub>0-∞</sub> and C<sub>max</sub> of repaglinide, which were adjusted for the expected effect of the SLCO1B1 521T>C polymorphism as derived from the previously published data. The calculated correction factors describing the mean expected magnitude of increase in the pharmacokinetic parameters in carriers of SLCO1B1 521TC and CC genotype compared with the TT genotype were 1.18 and 2.12 for AUC<sub>0-∞</sub> and 1.17 and 1.79 for C<sub>max</sub> of repaglinide, respectively. However, in this evaluation, no signifi-

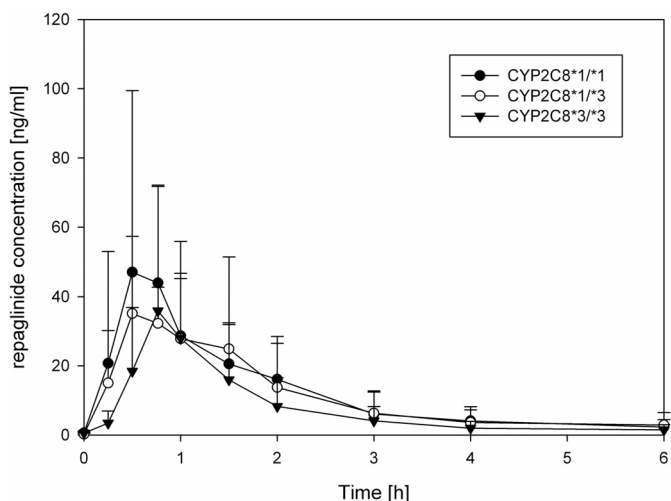


FIG. 1. Plasma concentration (mean and S.D.) of repaglinide up to 6 h after administration of 2 mg of repaglinide according to the CYP2C8\*3 genotype.

TABLE 2

Pharmacodynamic parameters of repaglinide relating to the *CYP2C8\*3* genotype

Data are shown as mean (95% confidence interval).

Genotype/Parameter	Insulin Concentration in Plasma		Blood Glucose Concentration	
	Mean Change	Maximal Change	Mean Change	Maximal Change
	mU/l		mM	
All subjects ( <i>n</i> = 29)	5.5 (3.7 to 7.2)	18.8 (12.6 to 25.0)	-0.53 (-0.71 to -0.34)	-1.69 (-1.97 to -1.41)
<i>CYP2C8*1/*1</i> ( <i>n</i> = 12)	6.7 (3.4 to 10.1)	23.9 (11.3 to 36.5)	-0.42 (-0.71 to -0.13)	-1.62 (-2.0 to -1.24)
<i>CYP2C8*1/*3</i> ( <i>n</i> = 13)	5.2 (2.7 to 7.8)	17.3 (9.1 to 25.6)	-0.65 (-0.97 to -0.30)	-1.83 (-2.37 to -1.28)
<i>CYP2C8*3/*3</i> ( <i>n</i> = 4)	2.6 (-1.2 to 6.3)	8.3 (-1.7 to 18.3)	-0.50 (-1.14 to -0.14)	-1.48 (-2.50 to -0.46)
<i>p</i> , one-way ANOVA	0.293	0.240	0.563	0.658
<i>p</i> , one-way ANOVA trend test (linear contrast)	0.125	0.105	0.783	0.763

cant effects of *CYP2C8\*3* on either pharmacokinetic parameter were observed ( $p > 0.05$ , one-way ANOVA with derived linear trend test).

### Discussion

In this study, we evaluated the potential impact of the *CYP2C8\*3* genotype on the pharmacokinetics and pharmacodynamics of repaglinide given in a therapeutically relevant dose of 2 mg and taking into account the *SLCO1B1 521T>C* genotype. Even if our results might suggest a higher *CYP2C8* metabolic activity in carriers of *CYP2C8\*3*, the observed effect was small and was not statistically significant. Moreover, we did not find any clear effect of the *CYP2C8\*3* polymorphism on insulin secretion and blood glucose-lowering activity. Thus, our data suggest that *CYP2C8\*3* has no clinical relevance in patients treated with repaglinide.

During the last decade, the important role of *CYP2C8* in oxidative metabolism of numerous drugs and endogenous agents such as arachidonic acid has been identified and prompted researchers to explore the relevance of the respective genetic polymorphisms and above all the role of *CYP2C8\*3* (Totah and Rettie, 2005; Daily and Aquilante, 2009). However, the available data on the impact of this variant are not unequivocal. For paclitaxel, a model *CYP2C8* substrate, a significantly reduced metabolic capacity relating to *CYP2C8\*3* was determined in several in vitro studies (Dai et al., 2001; Soyama et al., 2001; Bahadur et al., 2002). However, the presence of this allele could not explain the observed interindividual variability in paclitaxel pharmacokinetics in clinical studies (Henningsson et al., 2005; Marsh et al., 2007). On the other hand, increased catalytic activity in carriers of *CYP2C8\*3* was observed for the two oral antidiabetic agents rosigli-

tazone and pioglitazone (Kirchheiner et al., 2006; Aquilante et al., 2008; Tornio et al., 2008).

In addition, for repaglinide the existing data on the impact of the *CYP2C8\*3* polymorphism are not fully consistent. The working group of Niemi (Niemi et al., 2003b) evaluated the possible impact of *CYP2C8\*3* in healthy white volunteers. After administration of repaglinide given in a single dose of 0.25 mg, these authors found that the mean AUC and  $C_{max}$  of the drug, 45% ( $p < 0.005$ ) and 39% ( $p < 0.05$ ), respectively, were lower in 6 carriers of the *CYP2C8\*1/\*3* genotype than in 19 carriers of the *CYP2C8\*1/\*1* genotype. At the same time, this evaluation was the first one to show a link between *CYP2C8\*3* and altered pharmacokinetics of a drug metabolized by *CYP2C8* in vivo. The role of *CYP2C8\*3* was then confirmed in another study by the same authors who determined lower mean values of the AUC (48%) and  $C_{max}$  (44%) ( $p < 0.05$ ) of repaglinide in 10 heterozygous carriers of this allele than in 41 noncarriers treated with a single dose of 0.25 mg of repaglinide (Niemi et al., 2005). The important finding is that the observed differences in the pharmacokinetic parameters were not accompanied by differences in the blood glucose-lowering effects of repaglinide between the respective *CYP2C8* genotypes. Of note also is the fact that the repaglinide dose tested in both studies was clearly subclinical. Bidstrup et al. (2006) also evaluated the role of the *CYP2C8\*3* polymorphism in the pharmacokinetics of repaglinide; however, they administered the drug in a clinically relevant dose of 2 mg. In this evaluation, the AUC and the maximal concentration of repaglinide did not differ significantly between 24 wild-type individuals and 12 carriers of the variant allele, one of whom was homozygous. To explain this inconsistency, Bid-

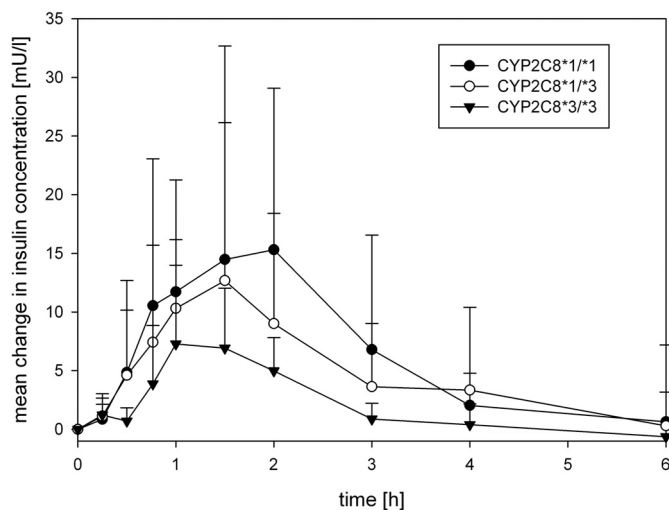


FIG. 2. Changes in insulin concentration (mean and S.D.) after administration of 2 mg of repaglinide up to 6 h postdose according to the *CYP2C8\*3* genotype.

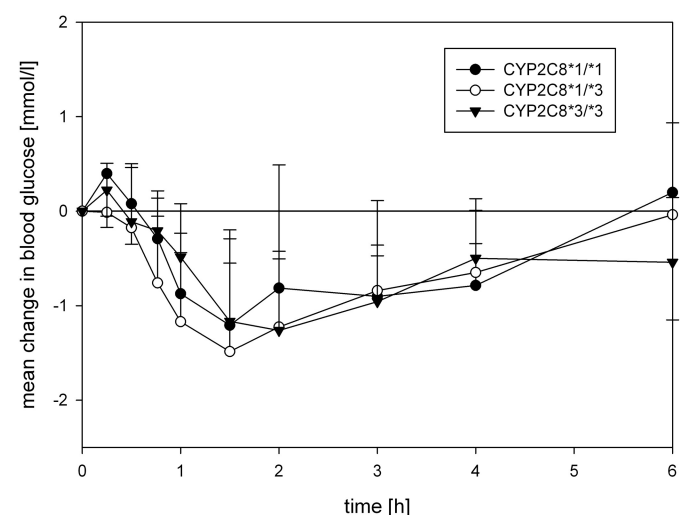


FIG. 3. Changes in blood glucose (mean and S.D.) after administration of 2 mg of repaglinide up to 6 h postdose according to the *CYP2C8\*3* genotype.

TABLE 3

Mean values for selected pharmacokinetic and pharmacodynamic parameters of repaglinide for combined CYP2C8\*3 and SLCO1B1 521T>C genotypes

Data are shown as mean and (S.D.). S.D. is given when data from at least three subjects were available; otherwise respective individual values are given.

Genotype CYP2C8/SLCO1B1 521T>C	CYP2C8*1/*1	CYP2C8*1/*3	CYP2C8*3/*3
<b>SLCO1B1 521TT</b>			
<i>n</i>	9	8	1
AUC <sub>0-∞</sub> (ng · ml <sup>-1</sup> · h)	92.3 (62.5)	117.1 (70.8)	115.5
C <sub>max</sub> (ng/ml)	52.5 (24.6)	57.0 (20.3)	36.6
Ae <sub>0-24 h</sub> of M1 (AU)	57.0 (80.9)	47.5 (28.2)	53.1
Ae <sub>0-24 h</sub> of M4 (AU)	181.7 (89.1)	226.4 (96.1)	79.4
Mean change in insulin (mM)	7.1 (5.1)	5.6 (4.1)	1.0
Mean change in glucose (mM)	-0.4 (0.5)	-0.5 (0.5)	-0.1
<b>SLCO1B1 521TC</b>			
<i>n</i>	3	4	2
AUC <sub>0-∞</sub> (ng · ml <sup>-1</sup> · h)	146.5 (141.5)	77.2 (21.1)	54.7,23.4
C <sub>max</sub> (ng/ml)	83.7 (88.3)	45.0 (17.3)	34.0,15.3
Ae <sub>0-24 h</sub> of M1 (AU)	39.9 (18.8)	33.3 (15.7)	52.8,78.0
Ae <sub>0-24 h</sub> of M4 (AU)	191.7 (120.7)	197.8 (125.2)	471.7,526.9
Mean change in insulin (mM)	5.7 (6.9)	5.6 (5.0)	1.5,1.7
Mean change in glucose (mM)	-0.5 (0.2)	-1.0 (0.6)	-0.2,-0.9
<b>SLCO1B1 521CC</b>			
<i>n</i>	0	1	1
AUC <sub>0-∞</sub> (ng · ml <sup>-1</sup> · h)		18.2	95.8
C <sub>max</sub> (ng/ml)		17.7	67.9
Ae <sub>0-24 h</sub> of M1 (AU)		41.4	29.1
Ae <sub>0-24 h</sub> of M4 (AU)		155.1	268.6
Mean change in insulin (mM)		0.6	6.1
Mean change in glucose (mM)		-0.2	-0.8

AUC<sub>0-∞</sub>, area under the plasma concentration time curve up to infinity; C<sub>max</sub>, maximal concentration; Ae<sub>0-24 h</sub>, amount excreted in urine up to 24 h postdose; AU, arbitrary units.

strup et al. (2006) speculated that the different doses of repaglinide given in the studies may be of importance, so that the contribution of the enzymes participating in the metabolism, CYP2C8 and CYP3A4, may differ with a varying dose of the drug.

In our project, the volunteers were also given a clinically relevant dose of repaglinide. However, in contrast to the previous studies, we covered a wide spectrum of parameters, including the evaluation of excretion of repaglinide metabolites M1 and M4 in the urine as well as pharmacodynamic parameters with glucose and insulin profiles. Moreover, we also involved a sufficient number of homozygous carriers of the CYP2C8\*3 variant in the study, so that the respective pharmacokinetic and dynamic parameters were calculated separately for subjects with none, one, and two variant alleles, to assess a possible trend attributable to the polymorphism. Despite the lack of statistical significance, most parameters indicate slightly higher metabolic activity related to the presence of CYP2C8\*3.

In addition to CYP2C8, Niemi et al. (2005) identified the polymorphism 521T>C in the SLCO1B1 gene, coding for the drug transporter organic anion-transporting polypeptide 1B1, to be an important factor accounting for much of the observed interindividual variability in repaglinide pharmacokinetics. They showed nearly 3-fold higher AUC values in homozygous carriers of the 521C variant than in those with the 521TT (wild-type) genotype. Moreover, the authors determined similar effects for different single doses of repaglinide, which varied from 0.25 to 2 mg, thus proving that the 521T>C polymorphism plays an important role in repaglinide pharmacokinetics irrespective of its dose (Kalliokoski et al., 2008b). The same authors also considered the potential impact of the observed pharmacokinetic differences between the respective SLCO1B1 genotypes on the glucose-lowering effect of the drug. However, the assessed tendency for a greater glucose decrease in carriers of the 521CC genotype was rather negligible and not significant (the maximum decrease in blood glucose was 2.3, 1.9, and 1.9 mM, respectively, in CC, TC, and TT genotype groups) (Kalliokoski et al., 2008a).

Thus, although we did not select participants according to the SLCO1B1 521T>C polymorphism, this genotype was also deter-

mined in our study population and taken into account in the evaluation of simultaneous effects of both polymorphisms, CYP2C8\*3 and 521T>C. However, neither in a two-way ANOVA considering both polymorphisms as factors nor in the evaluation of repaglinide AUC and C<sub>max</sub> corrected for the expected effect of the 521T>C polymorphism was there a relevant impact of CYP2C8\*3 with respect to the pharmacokinetic or dynamic parameters of repaglinide observed. Although these evaluations further support the negligible role of CYP2C8\*3 in repaglinide disposition, the lack of effect of the SLCO1B1 521T>C genotype was surprising. It has to be acknowledged that this study was not designed to detect differences with respect to the SLCO1B1 genotype. In fact, only two subjects were homozygous carriers of the 521C variant and both were simultaneously carriers of at least one CYP2C8\*3 allele, which may have an opposite effect on repaglinide disposition to the 521C variant. In contrast, only noncarriers of CYP2C8\*3 were included in most previous studies on the impact of the 521T>C polymorphism on repaglinide pharmacokinetics and dynamics (Kalliokoski et al., 2008a,b). Thus, the possibility that the effect of the 521T>C polymorphism on repaglinide pharmacokinetics may be apparent only if studied in clearly defined subpopulations cannot be ruled out.

In summary, the present study supports the view that the CYP2C8\*3 polymorphism does not play an important role in the pharmacokinetics and pharmacodynamics of repaglinide when a therapeutic dose of the drug is given. Therefore, it is not expected to be relevant for the safety of diabetic patients treated with the drug and the efficacy of the therapy. At the same time, the reasons for and importance of the unequal impact of CYP2C8\*3 on the disposition of various drugs are worth detailed examination in the future. For a more exact quantitative estimation of the effects of both CYP2C8 and SLCO1B1 and their mutual influence, larger studies with sufficient numbers of individuals in the respective subgroups are required, but the expected clinical relevance of this information would remain limited.

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**Authorship Contributions**

Participated in research design: Fuhr and Stingl.

Conducted experiments: Tomalik-Scharte, Fuhr, Doroshenko, Jetter, and Stingl.

Performed data analysis: Tomalik-Scharte, Hellmich, and Stingl.

Wrote or contributed to the writing of the manuscript: Tomalik-Scharte, Fuhr, and Stingl.

Other: Frank performed analysis of the drug and its metabolites.

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