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The effect of the *CYP2C8* genotype on the pharmacokinetics and pharmacodynamics of repaglinide.

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A list of nonstandard abbreviations used in the paper: $AUC_{0-\infty}$, area under the plasma concentration time curve up to infinity; Ae_{0-24h}, amount excreted in urine up to 24 h post-dose; C_{max}, maximal concentration, AU, arbitrary units, LC-MS/MS, liquid chromatography-tandem mass spectrometry; CI, confidence interval

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Abstract

The pharmacokinetics of repaglinide is subject to a pronounced inter-individual 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 Caucasians carrying *CYP2C8*3/*3* (n=4), *CYP2C8*1/*3* (n=13) or *CYP2C8*1/*1* (n=12). Following 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 hours post-dose. Repaglinide and the metabolites were quantified by LC-MS/MS. Considering only the effect of *CYP2C8*3*, the mean (95%CI) $AUC_{0-\infty}$ of repaglinide was 72.4 (6.7–138.0), 97.2 (59.2–135.2), 105.9 (52.4-159.3) $ng*mL^{-1}*h$ and C_{max} 38.5 (3.8–73.2), 50.3 (37.5-63.0), 60.3 (31.5-89.1) $ng*mL^{-1}$, respectively, in carriers of *CYP2C8*3/*3*, *CYP2C8*1/*3* and *CYP2C8*1/*1* (*p>0.05*, one-way ANOVA). Also, 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 dynamics were observed when AUC and C_{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.

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Introduction

Repaglinide, a meglitinide analogue, is a fast-acting insulin secretagogue drug used to normalize postprandial hyperglycemia in patients with type 2 diabetes. Following oral administration it is rapidly absorbed with absolute bioavailability of about 60% (Hatorp, 2002). Repaglinide is extensively biotransformed in the liver to inactive metabolites, which are predominantly excreted via the faeces and to about 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, while 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 (AUC) of repaglinide by 40%, while the CYP2C8 inhibitors trimethoprim and gemfibrozil led to an increase in AUC of the drug by about 60% and 700%, respectively (Niemi et al., 2001; Niemi et al., 2003a; Niemi et al., 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 Arg139Lys and Lys399Arg, is quite common in Caucasians, showing a frequency of about 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). As *CYP2C9*2* is associated to a reduced enzymatic activity to most substrates and there is some overlap between substrate recognition for CYP2C8 and CYP2C9, this linkage may contribute to a clinical relevance of the *CYP2C8*3*

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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; Green et al., 2009). However, the results are partly conflicting and no well-defined impact on CYP2C8 activity has been identified. Similarly, there is no consistent evidence for the role of *CYP2C8*3* in the metabolism of repaglinide. Niemi et al. found decreased (by 45 to 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 (Niemi et al., 2003b; Niemi et al., 2005). On the other hand, the study by Bidstrup et al. yielded no relevant differences in repaglinide pharmacokinetics with respect to this polymorphism (Bidstrup et al., 2006).

At the same time, the effects of polymorphisms in *SLCO1B1*, the gene encoding the organic anion transporting polypeptide 1B1 (OATP1B1), 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 Caucasians is 521T>C (allelic frequency 14%) resulting in the amino acid substitution Val174Ala 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 as compared to the 521TT genotype (Niemi et al., 2005; Kalliokoski et al., 2008b; Kalliokoski et al., 2008a).

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.

Methods:

Subjects.

Twenty-nine Caucasian 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. Having regard to the previously published data on the pharmacokinetic variability of repaglinide (Hatorp, 2002), a sample size of at least 3 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 about 7 o'clock in the morning after a 9 hour fast. The subjects continued fasting for 6 hours after taking the study medication. Furthermore, they were asked to abstain from alcohol, caffeine containing beverages and grapefruit products from 3 days before till 2 days after the dosing. Blood sampling for the determination of repaglinide concentration was carried out 3 min prior to the dosing and at 0:15, 0:30, 0:45, 1:00, 1:30, 2:00, 3:00, 4:00, 6:00, 8:00, 10:00, 12:00, 16:00, 24:00 hours post-dose (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 mmol/L (Accutrend® sensor, Hestia, Mannheim, Germany), additionally samples for insulin measurement were drawn up to 6 hours post-dose. Moreover, urine samples for quantification of repaglinide and its metabolites M1 and M4 were collected just prior to the dosing, and in intervals from 0 − 4, 4 − 8, 8 − 12, 12 − 16, and 16 − 24 h. For safety evaluation, adverse events and well-being were surveyed by non-leading questions asked at

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each time of blood sampling.

Analytical Methods

Repaglinide and its metabolites M1 and M4 were determined using LC-MS/MS according to previously published methods (Ho et al., 2004; Kajosaari et al., 2005b). Repaglinide was measured in plasma and urine, whereas both metabolites were determined in urine only due to their undetectable concentrations in plasma.

Quantification of repaglinide was carried out using peak area ratios of the analyte and the internal standard (diclofenac). Since 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 µg/L [LLOQ] and 79.139 µg/L and for urine samples between 0.394 µg/L [LLOQ] and 161.787 µg/L. Precision ranged from 7.5 % to 9.4 % in plasma and from 4.8 % to 10.7 % in urine while 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-labelled chemiluminescent immunometric assay (Insulin IMMULITE 2000, Siemens Healthcare Diagnostics GmbH) with a calibration range of 2 – 300 mU/l.

Genotyping methods

Genotyping for *CYP2C8*3* was performed as previously described (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 determination of the *SLCO1B1* 521T>C polymorphism was carried out 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[TM] version 1.5 (Pharsight, Mountain View, CA, USA) in the noncompartmental mode. Maximal plasma concentrations of repaglinide were obtained directly from the measured values. 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 hours post-dose (Ae_{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 hours post-dose and the baseline values, were considered. All calculations were performed using Excel 2003 (Microsoft corp., Seattle, WA, USA).

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 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

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correction factor reflecting an assumed mean increase of these parameters due to the presence of one or two 521C alleles as 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, following the administration of a single dose of 0.25 to 2 mg of repaglinide.(Niemi et al., 2005; Kalliokoski et al., 2008a; Kalliokoski et al., 2008b). All statistical calculations were done by SPSS 14.0 (SPSS Inc., Chicago, IL, USA).

Results:

Of 29 study participants, 15 were women and 14 men. The mean ±SD age was 35 ±12.7 years (range: 24-64 years), and the body mass index was 23.4 ±2.5 kg/m^2 (range: 18.6-29.3 kg/m^2). 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 studied population there was a complete linkage between *CYP2C8*3* and *CYP2C9*2*. With respect to the *SLCO1B1* 521T>C genotype, 18 subjects were homozygous wild-types, 9 were heterozygous and 2 were homozygous carriers of the variant allele.

All participants completed the study. Following the administration of repaglinide up to the end of the fasting time (6 hours post-dose), blood glucose ranged from 2.1 to 8.2 mmol/L. In 7 subjects, transient symptoms of hypoglycemia, such as dizziness, sweating and tremor were observed, but quickly disappeared after administration of 200 - 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 intervals for plasma and urine pharmacokinetic parameters of repaglinide in all study participants and stratified according to the *CYP2C8* genotype. Although the mean values of AUC$_{0-\infty}$ and C$_{max}$ 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 hours post-dose in carriers of the *CYP2C8*1/*1*, *1/*3* and *3/*3* genotypes is shown in Figure 1.

Similarly, taking into account repaglinide metabolites excreted in urine up to 24 hours, the mean amount of M4, but not M1, as well as the ratio of M4 to AUC$_{0-\infty}$ of repaglinide (data not shown) increased with the number of *CYP2C8*3* alleles. However, the observed trend was not statistically significant either.

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Pharmacodynamic results

Considering the mean change in insulin concentration and the maximal observed change in insulin concentration up to six hours post-dose, the time when lunch was given, there was an apparent tendency towards a lower insulin increase in the groups with at least one variant allele, suggesting a gen-dose dependent manner, see Table 2 and Figure 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) since the biggest glucose decrement was observed in carriers of the *CYP2C8*1/*3* genotype (Table 2, Figure 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) due to hypoglycemia related adverse events. None of the four subjects with the *CYP2C8*3/*3* genotype suffered from hypoglycemia.

*Assessment of concomitant effects of the CYP2C8*3 and SLCO1B1 521T>C polymorphisms*

To asses the potential concomitant effect of the *SLCO1B1* 521T>C polymorphism on the repaglinide pharmacokinetics and dynamics, a two-way ANOVA with both polymorphisms as factors was performed. Respective mean values and corresponding SD for resulting genotype groups are shown in Table 3. Due to the relatively small number of subjects treated in the study, none of the carriers of *CYP2C8*1/*1* had concurrently the 521CC genotype and in several genotype combination groups (*CYP2C8*1/*3* and 521CC, *CYP2C8*3/*3* and 521TT, *CYP2C8*3/*3* and 521CC) only one subject was identified in the studied population. Whereas for carriers of the 521TC genotype a trend was present of decreasing values in $AUC_{0-\infty}$, C_{max} and the mean change in insulin, and increasing values in the amount of M4 excreted, with the number of the *CYP2C8*3* alleles, no such trend was observed for 521TT and CC genotypes. However, except for a significant main effect of *SLCO1B1* on the amount

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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 $AUC_{0-\infty}$ and C_{max} 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 of the pharmacokinetic parameters in carriers of *SLCO1B1* 521TC and CC genotype as compared with the TT genotype were, respectively, 1.18 and 2.12 for $AUC_{0-\infty}$ and 1.17 and 1.79 for C_{max} of repaglinide. However, also in this evaluation, no significant 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. Consequently, 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 like arachidonic acid has been identified and prompted researchers to explore the relevance of the respective genetic polymorphisms, 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, an increased catalytic activity in carriers of *CYP2C8*3* was observed for the two oral antidiabetic agents rosiglitazone and pioglitazone (Kirchheiner et al., 2006; Aquilante et al., 2008; Tornio et al., 2008).

Also, for repaglinide the existing data on the impact of the *CYP2C8*3* polymorphism are not fully consistent. The working group around Niemi evaluated the possible impact of *CYP2C8*3* in healthy Caucasian volunteers. Following administration of repaglinide given in a single dose of 0.25 mg, the authors found the mean AUC and C_{max} of the drug, 45% (p<0.005) and 39% (p<0.05) respectively, lower in six carriers of the *CYP2C8*1/*3* genotype than in 19 carriers of the *CYP2C8*1/*1* genotype (Niemi et al., 2003b). At the same time, this evaluation was the first one to show a link between *CYP2C8*3* and altered pharmacokinetics

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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 non-carriers treated with a single dose of 0.25 mg of repaglinide (Niemi et al., 2005). The important thing is, that the observed differences in the pharmacokinetic parameters were not accompanied by differences in blood-glucose lowering effects of repaglinide between the respective *CYP2C8* genotypes. Of note is also the fact that the repaglinide dose tested in both studies was clearly subclinical. Bidstrup and colleagues also evaluated the role of the *CYP2C8*3* polymorphism in the pharmacokinetics of repaglinide, though, they administered the drug in a clinically relevant dose of 2 mg (Bidstrup et al., 2006). Interestingly, 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, Bidstrup and colleagues 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, in order to assess a possible trend attributable to the polymorphism. Despite the lack of statistical significance, most parameters indicate a slightly higher metabolic activity relatable to the presence of *CYP2C8*3*.

In addition to CYP2C8, Niemi and co-workers identified the polymorphism 521T>C in the *SLCO1B1* gene, coding for the drug transporter OATP1B1, to be an important factor accounting for much of the observed interindividual variability in repaglinide

pharmacokinetics. They showed nearly three-fold higher AUC values in homozygous carriers of the 521C variant than in those with the 521TT (wild type) genotype (Niemi et al., 2005). 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 to greater glucose decrease in carriers of the 521CC genotype was rather negligible and not significant (maximum decrease in blood glucose was 2.3, 1.9 and 1.9 mmol/L, 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 determined 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_{max} corrected for the expected effect of the 521T>C polymorphism was a relevant impact of *CYP2C8*3* with respect to the pharmacokinetic or dynamic parameters of repaglinide observed. Whereas 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. Actually, 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 non-carriers 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; Kalliokoski et al., 2008b). Thus, it cannot be ruled out that the effect of the 521T>C polymorphism on repaglinide pharmacokinetics may be apparent only if studied in clearly defined subpopulations.

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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, exploring the reasons for and importance of the unequal impact of *CYP2C8*3* on the disposition of various drugs is worth a 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: Julia Stingl, Uwe Fuhr

Performed the clinical study: Dorota Tomalik-Scharte, Oxana Doroshyenko, Alexander Jetter,

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Contributed to the writing of the manuscript: Dorota Tomalik-Scharte, Uwe Fuhr, Julia Stingl

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Footnotes

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Figure legends

Figure 1

Plasma concentration (mean and SD) of repaglinide up to 6 hours after administration of 2 mg of repaglinide according to the *CYP2C8*3* genotype.

Figure 2

Changes in insulin concentration (mean and SD) following administration of 2 mg of repaglinide up to 6 hours post-dose according to the *CYP2C8*3* genotype.

Figure 3

Changes of blood glucose (mean and SD) following administration of 2 mg of repaglinide up to 6 hours post-dose according to the *CYP2C8*3* genotype.

Tables

Table 1

Pharmacokinetic parameters of repaglinide and urinary excretion of repaglinide metabolites

M1 and M4 relating to the *CYP2C8*3* genotype

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

Data, obtained by noncompartmental analysis, are shown as mean and (95% confidence interval). $AUC_{0-\infty}$, area under the plasma concentration time curve up to infinity; C_{max}, maximal concentration; $t_{1/2}$, terminal half-life; time to reach maximal plasma concentration; Ae_{0-24h}, amount excreted in urine up to 24 h post-dose, AU, arbitrary units.

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Table 2

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

Genotype/Parameter	Mean change in insulin concentration in plasma (mU l^{-1})	Maximal change in insulin concentration in plasma (mU l^{-1})	Mean change in blood glucose concentration (mmol l^{-1})	Maximal change in blood glucose concentration (mmol l^{-1})
All subjects (n = 29)	5.5 (3.7 – 7.2)	18.8 (12.6 – 25.0)	-0.53 (-0.71 - -0.34)	-1.69 (-1.97 – -1.41)
CYP2C8*1/*1 (n = 12)	6.7 (3.4 – 10.1)	23.9 (11.3 – 36.5)	-0.42 (-0.71 - -0.13)	-1.62 (-2.0 - -1.24)
CYP2C8*1/*3 (n = 13)	5.2 (2.7 – 7.8)	17.3 (9.1 – 25.6)	-0.65 (-0.97 - -0.30)	-1.83 (-2.37 - -1.28)
CYP2C8*3/*3 (n = 4)	2.6 (-1.2 – 6.3)	8.3 (-1.7 – 18.3)	-0.50 (-1.14 - -0.14)	-1.48 (-2.50 - -0.46)
***P* value one-way ANOVA**	0.293	0.240	0.563	0.658
***P* value one-way ANOVA trend**	0.125	0.105	0.783	0.763

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

Table 3

Mean values for selected pharmacokinetic and pharmacodynamic parameters or repaglinide

for combined *CYP2C8*3* and *SLCO1B1* 521T>C genotypes

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

Data are shown as mean and (SD); SD was given when data from at least three subjects were available, otherwise respective

individual values are given; $AUC_{0-\infty}$, area under the plasma concentration time curve up to infinity; C_{max}, maximal concentration;

Ae_{0-24h}, amount excreted in urine up to 24 h post-dose, AU, arbitrary units;
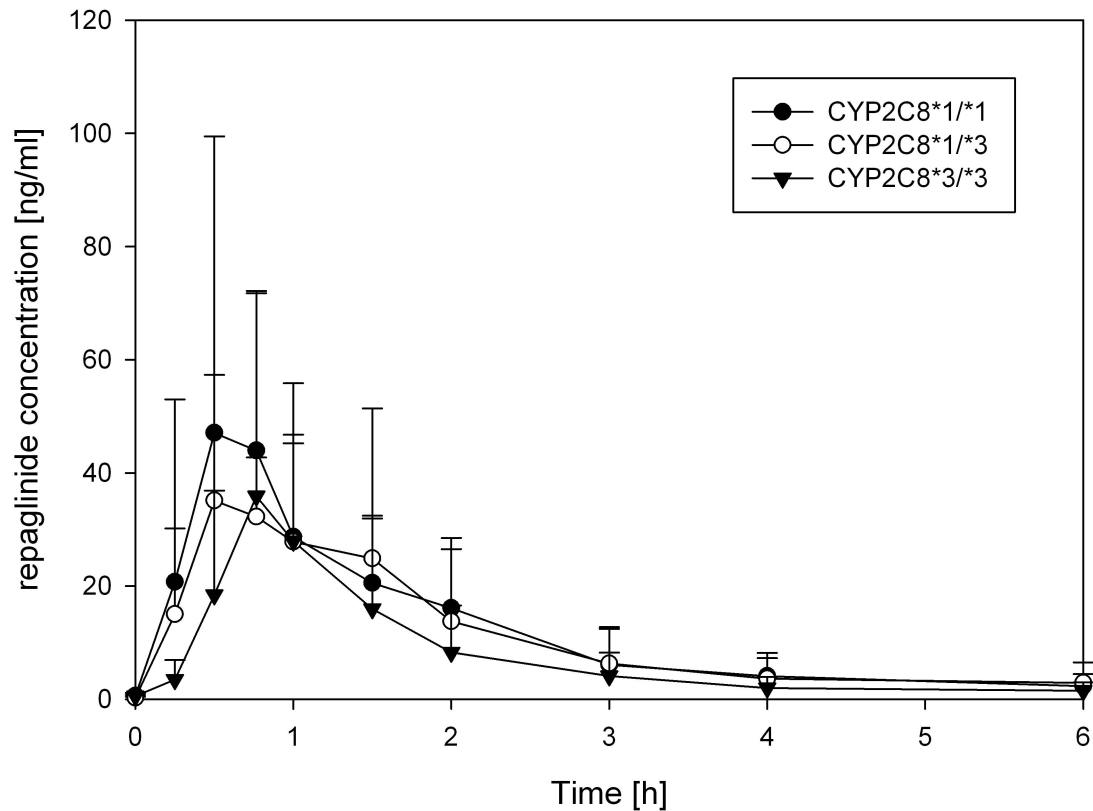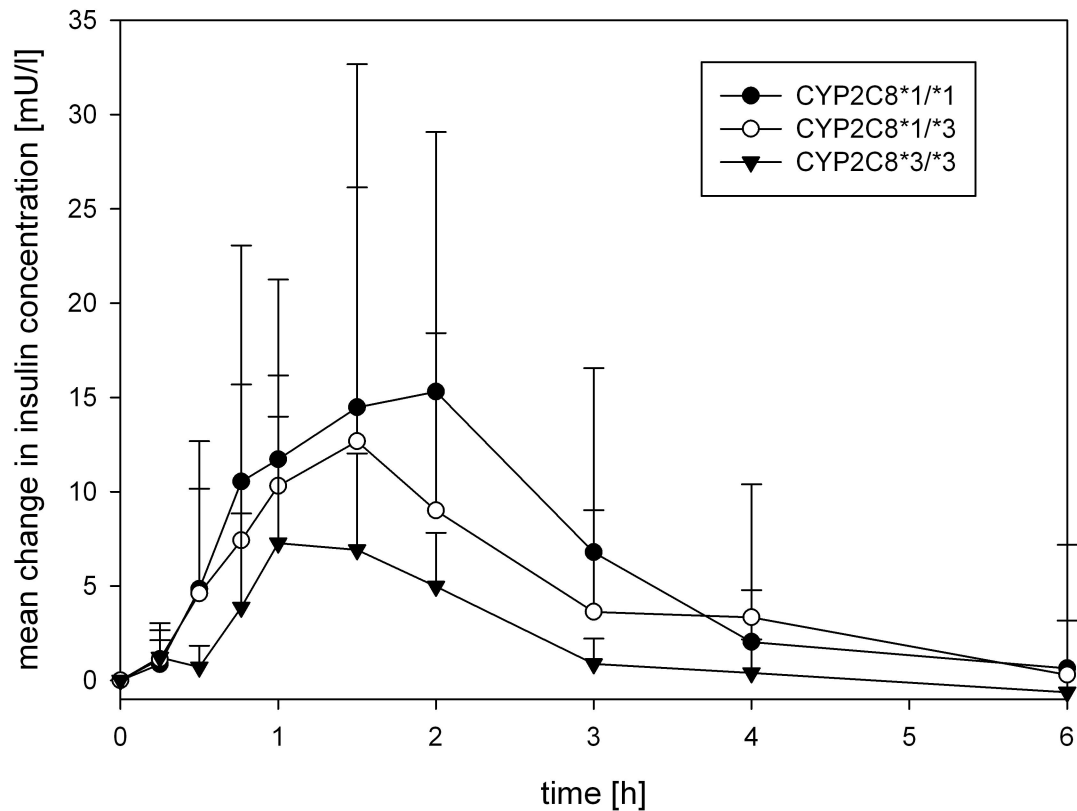
Figure 1

Figure 2

Figure 3