Int J Legal Med (2010) 124:187–194
DOI 10.1007/s00414-010-0418-8

ORIGINAL ARTICLE

An in vitro approach to estimate putative inhibition of buprenorphine and norbuprenorphine glucuronidation

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Received: 25 August 2009 / Accepted: 5 January 2010 / Published online: 29 January 2010
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Abstract An in vitro inhibition study was performed to investigate potential drug–drug interactions on glucuronidation of buprenorphine (BUP) and norbuprenorphine (NBUP), which represents the major elimination pathway of the drug using cDNA-expressed uridine 5′-diphosphate glucuronosyltransferases (UGTs) and human liver microsomes (HLMs). Following identification of major UGT enzymes for BUP and NBUP glucuronidation, substrates were incubated with drugs (amitriptyline, nortriptyline, lamotrigine, oxazepam, and temazepam), which are extensively cleared by glucuronidation as well as are often used during maintenance treatment. To evaluate the inhibitory potential, the half maximal inhibitor concentration (IC_{50}), the inhibition constant (K_i), and the inhibitor concentration (K_I) that yield half the maximum rate of inactivation and the enzyme inactivation rate constant (k_{inact}) were determined, if appropriate. Amitriptyline and temazepam are inhibitors of NBUP glucuronidation (UGT1A3, HLMs), whereas BUP glucuronidation was affected by amitriptyline (HLMs), oxazepam, and temazepam (UGT2B7). Additionally, BUP inhibits NBUP glucuronidation (UGT1A1, 1A3, HLMs) and vice versa (UGT1A3). A decrease in the metabolic clearance of NBUP may increase the risk of adverse effects such as respiratory depression. Further investigations are needed to evaluate whether inhibition of BUP and NBUP glucuronidation contributes to adverse events.

Keywords Buprenorphine · Norbuprenorphine · Glucuronidation · Inhibition

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Introduction

Buprenorphine (BUP) is a semisynthetic, highly lipophilic opioid derivative of the alkaloid thebaine. A preliminary communication of agonist and antagonistic effects was already given in 1972, but even to date the mechanisms of action of BUP are not fully understood. The drug has been described as a partial agonist at the mu receptor and can also bind to kappa and delta opioid receptors. It blocks epsilon receptors at low doses and has recently been shown to interact with the ORL-1 receptor [1].

BUP is used in low doses of 0.3–0.6 mg for pain management and has been approved for the treatment of opioid dependency with an acceptable daily intake of up to 32 mg [2, 3]. The use of BUP in the treatment of opioid addicts has increased significantly over the last years [4]. Co-morbidity in opioid dependency often leads to simultaneous treatment with psychotropic drugs. Not only benzodiazepines but also mood stabilizers and antidepressants such as lamotrigine or amitriptyline are additionally prescribed or used illicitly, of which some may interact with BUP metabolism [5].

BUP is rapidly metabolized by *N*-dealkylation to norbuprenorphine (NBUP) through cytochrome P450 (CYP) 3A4 and 2C8 [6]. NBUP is likely to contribute to the pharmacology of BUP acting as a full, but less active agonist at the mu opioid receptor [1]. While BUP is less prone for respiratory depression, this crucial adverse effect has been observed for NBUP [7]. BUP and NBUP are cleared primarily by glucuronidation through uridine 5′-diphosphate glucuronosyltransferases (UGTs) 1A1, 1A3, and 2B7 or UGT1A1 and 1A3 to produce buprenorphine-3-β-D-glucuronide (BUPG) or norbuprenorphine-3-β-D-glucuronide (NBUPG), respectively [8, 9].

The concentration range of BUP observed during maintenance treatment largely overlaps with that in BUP-related death cases. However, fatalities involving BUP alone are a particular rare occurrence [10]. There is limited evidence of drugs influencing the kinetics of BUP through modulation of CYP3A4 [2], but there is no information at all whether concomitantly used drugs will interact with BUP or NBUP glucuronidation.

The high dosages used in maintenance therapy allows for in vitro rather than in vivo studies. First, recombinant UGT enzymes were screened for their activity toward BUP and NBUP. Then, five psychotropic drugs frequently used during BUP treatment and predominantly cleared by glucuronide conjugation were investigated for their possible influence on BUP and NBUP conversion. These in vitro data should help to evaluate the likelihood of drug–drug interaction in individuals during BUP maintenance therapy.

Materials and methods

Enzymes and chemicals

UGT reaction mix solution A (25 mM uridine 5′-diphospho-glucuronic acid (UDPGA) in water), UGT reaction mix solution B (250 mM Tris–HCl, 40 mM $MgCl_2$, 0.125 mg/mL alamethicin in water), pooled human liver microsomes (HLMs, n=30), and human supersomes (UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B15, and control supersomes) were obtained from NatuTec (Frankfurt, Germany). Supersomes are microsomes (recombinant cDNA-expressed enzymes) prepared from baculovirus-infected insect cells.

BUP hydrochloride, amitriptyline, nortriptyline, lamotrigine, oxazepam, and temazepam were purchased from Sigma/Aldrich (Steinheim, Germany). NBUP, NBUPG, BUP-d_4, and NBUP-d_3 as standard solutions were provided by LGC Standards (Wesel, Germany); BUPG was supplied by ElSohly Laboratories (Oxford, MS, USA); acetonitrile and methanol were obtained from Roth (Karlsruhe, Germany).

Measurement of glucuronide formation by high-pressure liquid chromatography/tandem mass spectrometry

Glucuronide concentrations were determined by high-pressure liquid chromatography/tandem mass spectrometry (LC-MS/MS, API 4000, Applied Biosystems, Darmstadt, Germany) with a TurboIon® ionization source operated in the positive ion mode. The mass analyzer was coupled to an Agilent model 1100 series LC system (binary pump and autosampler, Agilent, Waldbronn, Germany). Chromatographic separation was achieved with a Phenomenex Luna column C18(2) (150×2.00 mm, Phenomenex, Torrance, CA, USA) using isocratic elution with 4 mM ammonium acetate buffer pH 3.2/acetonitrile/methanol (60:32:8 by vol.) at a flow rate of 0.25 mL/min.

BUPG and NBUPG were stable under the reaction conditions used, during sample processing and in processed samples for 48 h, at least. Formation of BUPG and NBUPG was linearly increasing with the incubation time (5–60 min) and the protein concentration (0.125–0.5 mg/mL). Detection of the glucuronides was linear at 1–50 (1.56–78 nM) and 5–100 ng/mL (8.5–170 nM) with lower limits of detections at 0.7 (1.1 nM) and 1.2 ng/mL (2 nM) for BUPG and NBUPG, respectively. Ion suppression/enhancement or carry over could not be observed. It was also confirmed that intra- and inter-day precision were $<15\%$ (n=8, 5, and 25 ng BUPG/mL (7.8 and 39 nM), 10 and 50 ng NBUPG/mL (17 and 85 nM)). Accuracy was 97.6% and 99.3% for BUPG and NBUPG, respectively.

Enzyme kinetics and screening of inhibition

First, UGTs responsible for BUPG and NBUPG formation were identified, and their Michaelis–Menten constant K_m, and the maximum reaction velocity (v_{max}) were determined.

For initial screening experiments, five potential inhibitors used during BUP medication assisted maintenance therapy and being substrates of UGTs involved in BUP metabolism were chosen including amitriptyline, nortriptyline, lamotrigine, oxazepam, and temazepam. In case of significant inhibition (at least 60% of the control velocity at the highest inhibitor concentration), further data points were established in order to accurately determine inhibitor concentrations corresponding to a 50% inhibition of substrate metabolism (IC_{50}) at a K_m concentration of BUP or NBUP, respectively. Determination was carried out with and without a 15-min preincubation step of each inhibitor to test for a possible mechanism-based inhibition.

The inhibition constant K_i was determined if similar results from experiments with or without a preincubation step suggested reversible inhibition. The inhibitor concentration K_I that yields half the maximum rate of inactivation and the enzyme inactivation rate constant k_{inact} were determined if there was evidence of a mechanism-based inhibition.

Kinetic assays to determine K_m and v_{max}

A typical incubation mixture—in a total volume of 200 μL—containing 50 mM Tris–HCl buffer pH 7.5, 8 mM $MgCl_2$, 25 μg/mL alamethicin, 2 mM UDPGA, and 0.25 mg/mL recombinant UGTs or HLMs and substrate was incubated at 37°C for 30 min in a shaking water bath. The reaction mixture was stopped by 150 μL ice-cold acetonitrile/glacial acetic acid and chilled on ice for 30 min,

and then 10 µL NBUP-d_3 (1 or 5 µg/mL for the determination of NBUPG and BUPG, respectively) was added. Following centrifugation at 19,000×g for 10 min, 10 µL of the supernatant fraction was injected into the LC-MS/MS system.

The following substrate concentrations have been used to determine the kinetic parameters: UGT1A1, 10–160 µM BUP and 10–150 µM NBUP; UGT1A3, 0.4–8 µM BUP and 5–100 µM NBUP; UGT2B7, 4–60 µM BUP; HLMs, 1–160 µM BUP and 2–120 µM NBUP. Samples were diluted before analysis to a concentration which was well within the calibration range. K_m and v_{max} were obtained by fitting experimental data to kinetic models using SigmaPlot 9.0 (Point Richmond, CA, USA). Goodness of fit was assessed from statistical parameters such as the Akaike information criterion and the coefficient of determination (R^2).

Essentially identical assays were performed to estimate K_m values of inhibitors at the following concentrations: 70–870 µM amitriptyline (UGT1A3 and HLMs), 80–2,500 µM lamotrigine (UGT1A3 and HLMs), 140–700 µM oxazepam (UGT2B7 and HLMs), and 60–2,000 µM temazepam (UGT1A3, UGT2B7, and HLMs).

Screening of inhibitory potencies

Incubation was performed at a substrate concentration corresponding to the K_m value determined above. All inhibitors were prepared in methanol and evaporated to dryness prior to addition into the incubation system. For inhibition screening, inhibitor concentrations were close to $1/2K_m$, K_m, and $2{\cdot}K_m$ for the respective enzyme. Then, additional data points were established for drugs inhibiting formation of BUPG and NBUPG (at least 60% of the control velocity at the highest inhibitor concentration). Experiments were performed with and without a 15-min preincubation step to test for a possible mechanism-based inhibition. Control enzymes and incubation mixtures without inhibitor were used as controls. All experiments were carried out in duplicate.

For experiments without preincubation, the incubation and assay conditions described previously were used. For reactions carried out with preincubation, incubation conditions were as described for reactions without preincubation except that BUP or NBUP was added after 15 min. Samples were further processed as described above.

BUPG and NBUG formation rate involving an inhibitor was expressed as a percentage ratio relative to the control velocity without inhibitor. IC_{50} values were determined by fitting experimental data to the expressions for competitive, non-competitive, or mixed inhibition using SigmaPlot 9.0 (Point Richmond) and assessing the goodness of fit from the coefficient of determination (R^2) using non-linear regression.

Determination of K_i values

The inhibitor constant (K_i) is an indication of how potent an inhibitor is; it is the concentration required to produce half maximum inhibition.

Experiments were performed at three different substrate concentrations ($1/2K_m$, K_m, and $2{\cdot}K_m$) and four different inhibitor concentrations ($1/4{\cdot}IC_{50}$, $1/2{\cdot}IC_{50}$, IC_{50}, and $2{\cdot}IC_{50}$), respectively, using the same incubation conditions as specified above. Assays were carried out in quadruplicate, and mean values were analyzed by Dixon plots [11], in which the reciprocal velocity ($1/v$) is plotted against the inhibitor concentration at each substrate concentration. The resultant series of straight lines for different substrate concentrations intersect at a point where the inhibitor concentration on the x-axis is equal to the negative of the K_i value. Also, such lines intersect above the x-axis for competitive inhibition, whereas they intersect on the x-axis for non-competitive inhibition.

Determination of K_I and k_{inact}

K_I and k_{inact} were determined in time-dependent two-step experiments at four different inhibitor concentrations ($1/4\,K_m$, $1/2K_m$, K_m, and $2K_m$) and at a substrate concentration of K_m. The primary incubation mixture (150 µL) contained the inhibitor, 50 mM Tris–HCl buffer, pH 7.5, 8 mM $MgCl_2$, 25 µg/mL alamethicin, 2 mM UDPGA, and 2 mg/mL recombinant UGTs or HLMs. Prior to and every 5 min during the incubation period (20 min), a 25-µL aliquot of the primary incubation mixture was transferred to a secondary incubation mixture (175 µL), which was made up of the respective free compounds at K_m concentration and the same components as the primary one. Incubation was then performed for 30 min at 37°C, and the sample was further processed for analysis as described above. Assays were carried out in quadruplicate, and mean values were analyzed by Kitz–Wilson plots [12], where rate constants for the inhibition reaction were initially obtained from the slope of a plot of the logarithm of the percentage of enzyme activity remaining as a function of time. Then, a plot of 1/slope as a function of 1/inhibitor concentration [I] yields the inverse of k_{inact} as the y intercept. The x intercept of this plot is the negative inverse of the affinity constant K_I.

Results

Kinetic data are summarized in Table 1. UGT1A1, 1A3, 2B7, and HLMs were involved in BUP glucuronidation following Michaelis–Menten kinetics, while UGT1A1, 1A3, and HLMs participated to NBUP glucuronidation

Table 1 Enzyme kinetics

Enzyme models	Substrate	Kinetics	K_m (μM)	v_{max} (pmolmin^{-1}mg^{-1})	v_{max}/K_m (μlmin^{-1}mg^{-1})
UGT1A1	Buprenorphine	MM	24.8	6,726.8	271.2
	Norbuprenorphine	SI	26.0	714.6	27.5
UGT1A3	Buprenorphine	MM	2.5	642.6	257.0
	Norbuprenorphine	SI	17.5	387.0	22.1
	Amitriptyline	Hill	417.3	n.d.	–
	Lamotrigine	Atypical	10.6	n.d.	–
	Temazepam	Atypical	82.0	n.d.	–
UGT2B7	Buprenorphine	MM	4.3	823.8	191.6
	Oxazepam	Hill	151.0	n.d.	–
	Temazepam	Hill	418.3	n.d.	–
HLM	Buprenorphine	MM	37.4	4,276.4	114.3
	Norbuprenorphine	SI	11.7	528.9	45.2
	Amitriptyline	MM	295.7	n.d.	–
	Lamotrigine	Hill	347.8	n.d.	–
	Oxazepam	MM	29.3	n.d.	–
	Temazepam	MM	209.2	n.d.	–

Enzyme models best-fit estimate, *MM* Michaelis–Menten model, *SI* substrate inhibition kinetics, *atypical* biphasic kinetics (nonasymptotic), *Hill equation* sigmoidial kinetics, K_m Michaelis–Menten constant, v_{max} maximum velocity, *n.d.* not determined, (–) not applicable

(Fig. 1); substrate inhibition was evident with UGT1A1 and 1A3 as well as with HLMs.

K_m values of lamotrigine and temazepam did not fit any kinetic model; however, their biphasic kinetic profile has two distinct phases at high and low substrate concentrations. Temazepam showed Hill kinetics at both low and high substrate concentrations. Glucuronidation of lamotrigine followed Michaelis–Menten kinetics at low and Hill kinetics at high concentrations (Fig. 2). K_m values estimated from the curved portion of the plot at lower substrate concentrations were considered for further experiments.

Inhibitory potencies determined with and without a preincubation step are summarized in Table 2. Nortriptyline being metabolized by neither recombinant UGTs nor pooled HLMs was not further tested. NBUP (UGT1A1 and HLMs), amitriptyline, lamotrigine, oxazepam (HLMs), and temazepam (UGT1A3 and HLMs) had basically no or little effect on the glucuronidation of BUP.

Referring to the results above, inhibition of BUPG formation by amitriptyline, oxazepam, temazepam, and NBUP was further specified (Table 3). As expected, the IC_{50} value of amitriptyline via UGT1A3 was lower following preincubation than without preincubation with regard to the formation of NBUPG. K_i was determined for NBUP, oxazepam, and temazepam and amitriptyline, suggesting no mechanism-based inhibition of BUPG formation. The Dixon plot for

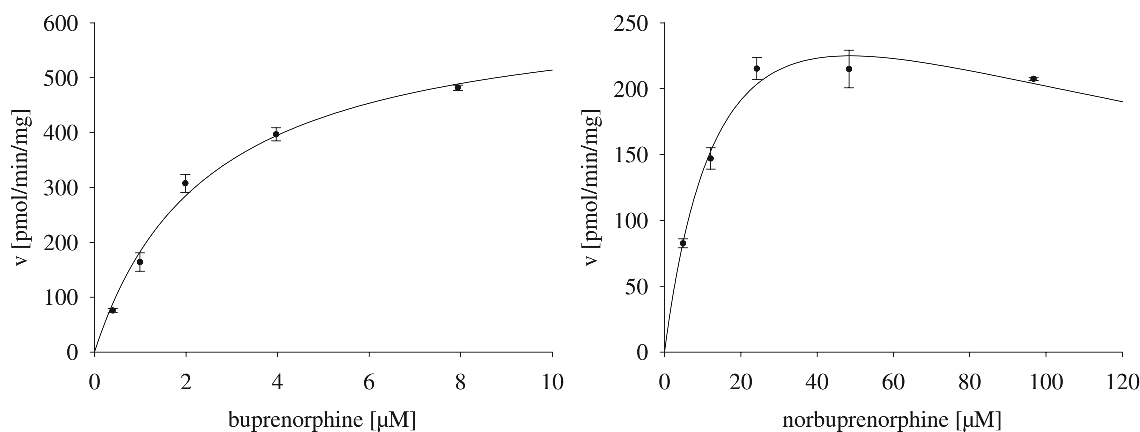

Fig. 1 Kinetics of buprenorphine (BUP) and norbuprenorphine (NBUP) glucuronidation. BUP showed Michaelis–Menten kinetics, NBUP showed substrate inhibition. BUP (0.4–8 μM) and NBUP (5–100 μM) were incubated with recombinant UGT1A3 (0.25 mg protein/mL) for 30 min at 37°C. Data represent mean values (n=2)

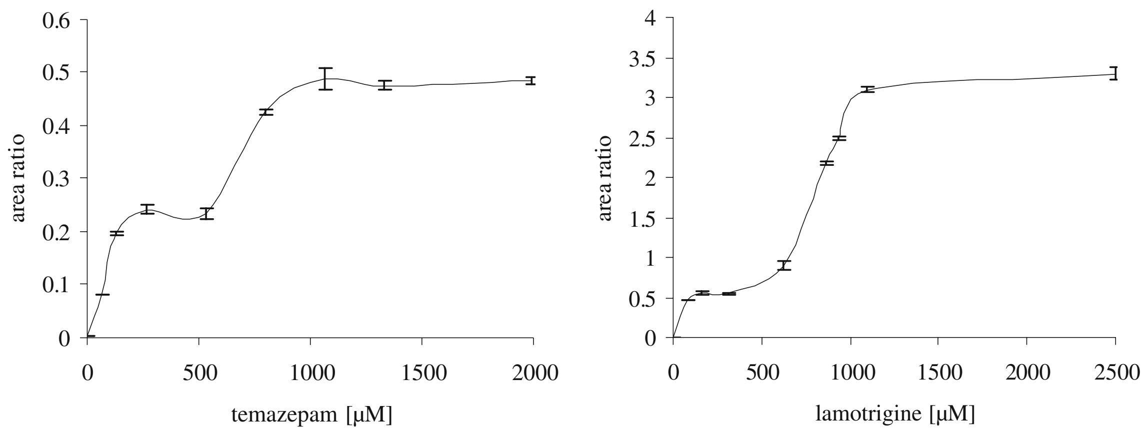

Fig. 2 Atypical kinetics of temazepam and lamotrigine. Temazepam (60–2,000 µM) and lamotrigine (80–2,500 µM) were incubated with recombinant UGT1A3 (0.25 mg protein/mL) for 30 min at 37°C. K_m values were estimated from the curved portion of the plot at lower substrate concentrations. Data represent mean values (n=2)

oxazepam (UGT2B7) is shown in Fig. 3. Reversible inhibition was also suggested for BUP, temazepam, and amitriptyline (HLMs) regarding NBUPG formation. A mechanism-based component in the inhibition of UGT1A3 activity was evident for amitriptyline (Fig. 4; Table 3).

Discussion

BUP is considered as a safe alternative to orally administered methadone in opioid dependent individuals because it is less likely to cause serious respiratory depression per se [13, 14]. Nevertheless, there is an increase in reports on dangerous and potentially fatal overdoses during the last few years [7, 15, 16]. Since NBUP is a major metabolite of BUP in vivo and its steady-state concentration is comparable or even exceeds that of BUP following administration by the sublingual route, it is likely to contribute to its pharmacological effects in vivo. Moreover, NBUP has been shown to induce respiratory depression that appears to be mediated by mu opioid receptors of the brain and also of the lungs [17]. Both the parent drug and its metabolite are

Table 2 Inhibition screening

Enzyme	Inhibitor	% Reaction velocity (glucuronide formation of BUP or NBUP, respectively), compared to the control without inhibitor					
		Without preincubation			With preincubation		
		$1/2K_m$	K_m	$2K_m$	$1/2K_m$	K_m	$2K_m$
Substrate buprenorphine							
UGT1A3	Norbuprenorphine	87	77	53	93	89	70
UGT2B7	Oxazepam	63	47	29	74	53	36
	Temazepam	55	34	38	71	72	52
HLMs	Amitriptyline	75	61	32	77	58	35
Substrate norbuprenorphine							
UGT1A1	Buprenorphine	60	56	51	75	62	63
UGT1A3	Buprenorphine	79	64	36	87	58	42
	Amitriptyline	70	55	23	54	39	16
	Temazepam	85	68	37	84	65	50
HLMs	Buprenorphine	74	58	38	70	50	33
	Amitriptyline	37	22	7	40	24	7
	Temazepam	63	57	49	78	75	52

Screening of selected psychotropic drugs on the glucuronidation of buprenorphine and norbuprenorphine at three different inhibitor concentrations ($1/2K_m$, K_m, and $2K_m$) with and without a preincubation step of 15 min at 37°C

IC_{50} inhibitor concentration that yields 50% of the control activity, K_m Michaelis–Menten constant

Table 3 Kinetic constants (IC_{50}, K_i, K_I, and k_{inact}) of the most potent inhibitors on buprenorphine glucuronide and norbuprenorphine glucuronide formation

Enzyme	Inhibitor	IC_{50} (μM) without preincubation	IC_{50} (μM) with preincubation	K_i (μM)	KI (μM); k_{inact} (1/min)
Substrate buprenorphine					
UGT1A3	Norbuprenorphine	40.5	53.6	1.9	n.d.
UGT2B7	Oxazepam	118.3	206.1	137.8	n.d.
	Temazepam	322.2	575.7	173.3	n.d.
HLMs	Amitriptyline	381.7	381.7	102.9	n.d.
Substrate norbuprenorphine					
UGT1A1	Buprenorphine	50.3	80.3	35.2	n.d.
UGT1A3	Buprenorphine	4.7	4.6	1.3	n.d.
	Amitriptyline	496.9	278.3	n.d.	571.9; 0.31
	Temazepam	137.6	143.1	154.3	n.d.
HLM	Buprenorphine	55.5	46.6	54.1	n.d.
	Amitriptyline	109.8	116.1	78.2	n.d.
	Temazepam	457.6	470.3	53.6	n.d.

A mechanism-based component was evident for amitriptyline

IC_{50} inhibitor concentration that yields 50% of the control activity, *K_i* inhibition constant, *K_I* inactivator concentration at which the inactivation rate is half of maximum, *k_{inact}* enzyme inactivation rate constant, *n.d.* not determined

subject to considerable glucuronidation. Therefore, inhibition of BUP or NBUP conjugation by mutual inhibition or co-administered drugs may result in decreased metabolic clearance and/or increased bioavailability [18].

Glucuronidation is a major pathway for the elimination of many xenobiotics and endogeneous compounds, and genetic polymorphism of UGT may be of toxicological and physiological importance. Genetic polymorphism has been described in almost all UGT family members with possibly altered enzyme function or expression and therefore with reduced drug clearance [19, 20]. To date, functional significance has only been convincingly demonstrated for genetic polymorphism of UGT1A1 leading to Gilbert and Crigler Najjar syndromes characterized by severe unconjugated hyperbilirubinemia.

The present investigation is considered as a first approach to study possible inhibitory interactions in BUP and NBUP conjugation.

The determination of in vitro kinetic constants may be used for a conservative in vitro–in vivo estimation of human pharmacokinetics and for predicting drug–drug interactions [21]. Assessing IC_{50} values allows to differentiate between reversible and irreversible inhibition. A more pronounced inhibition following preincubation of the

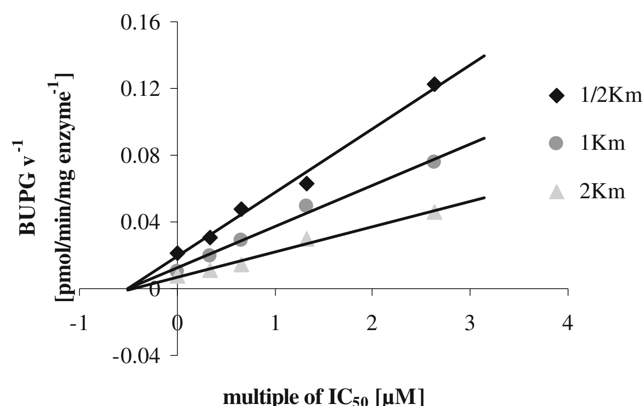

Fig. 3 Dixon plot (n=4) of the competitive inhibition of UGT2B7-mediated buprenorphine glucuronidation by temazepam. The glucuronide formation (BUPG) in the presence of temazepam is expressed as pmol $min^{-1} mg^{-1}$ enzyme versus inhibitor concentration, which is a multiple of its IC_{50} value

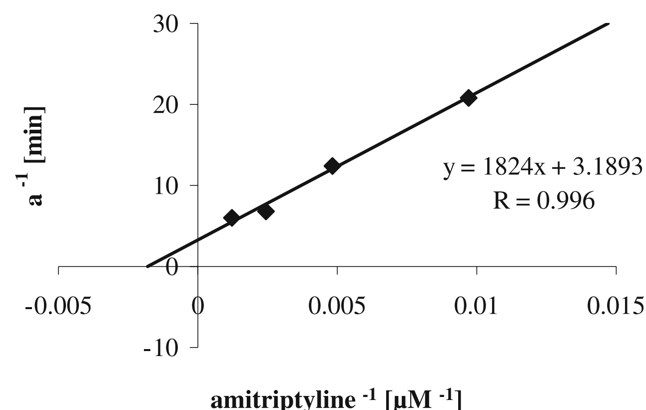

Fig. 4 Kitz–Wilson plot of amitriptyline (UGT1A3); *a* gradient of the linear equations established from experiments at four different inhibitor concentrations. 40 μM: $y=-0.0479x+1.0829$; $R=0.8666$. 80 μM: $y=-0.0808x+1.1512$; $R=0.7793$. 160 μM: $y=-0.1478x+1.2914$; $R=0.7193$. 320 μM: $y=-0.1683x+1.2985$; $R=0.8276$. *R* coefficient of correlation

inhibitor prior to addition of the substrate is indicative of irreversible inactivation [22]. An ongoing and still unresolved challenge is the interpretation and the comparison of kinetic inhibitory constants determined with UGT inhibitors [23]. Mostly, evaluation is made following those for CYP enzymes, which are well known and where in vitro tools and processes are well developed. Referring to this proposal, compounds with IC_{50} or $K_i \geq 30$ µM are not assumed to cause severe drug–drug interactions. Drugs with respective values ≤1 µM are assumed to have some potential of drug interaction in vivo. If a drug is identified as a mechanism-based inhibitor, however, IC_{50} or K_i values greater than 30 µM might be of great concern because many drugs that cause serious drug–drug interactions belong to this type of inhibitors [22].

The recent experiments revealed a major involvement of UGT1A1, 1A3, and 2B7 in BUP glucuronidation, whereas UGT1A1 and 1A3 play a role in NBUP metabolism. Comparison of the v_{max}/K_m ratios (Table 1) suggests little difference in UGT preference. Considering BUP glucuronidation first, both oxazepam and temazepam showed a decreased inhibitory potency toward BUPG formation by UGT2B7 following preincubation, which may be explained by inhibitor consumption due to metabolism during preincubation [22, 24]. Inhibition was reversible, and IC_{50} as well as K_i values was far above 30 µM for both compounds. Also, no significant inhibition of BUPG formation was detected with oxazepam and temazepam via HLMs. Reportedly, lower K_m values of substrates and inhibitors using recombinant UGT2B7 instead of HLMs have been attributed to a lower content of inhibitory unsaturated long-chain fatty acids in the expression system [25]. The present results, however, did not support this finding.

Interestingly, NBUP itself seems to inhibit conjugation of BUP by UGT1A3. Inhibition by NBUP was stronger than by oxazepam or temazepam, and a K_i value of 1.9 µM indicates a high affinity toward the enzyme. Consequently, formation of NBUP over BUPG may be favored. Previous studies revealed the mean concentration of BUPG to be lower than that of the parent drug and NBUPG to be the major metabolite in blood under steady-state conditions [3, 26]. The present results may account for these findings, in part. On the contrary, the inhibitory impact of NBUP on the glucuronidation of BUP via UGT1A1 and HLMs was insignificant.

The inhibition potency of amitriptyline toward NBUPG formation mediated by UGT1A3 was increased following preincubation (Table 3). Results suggest a mechanism-based component in the inhibition of the glucuronidation of NBUP by amitriptyline via UGT1A3. This finding is in accordance with the results of Hara et al. [27], where tricyclic antidepressants such as amitriptyline or clomipramine strongly inhibited UGT1A3-mediated glucuronidation of morphine. Inhibition was assigned as non-competitive (morphine-3-glucuronide) or of mixed inhibitory type (morphine-6-glucuronide), but was not further characterized. Amitriptyline showed a strong inhibitory potency when HLMs were used, but there was no evidence for a mechanism-based inhibition. NBUPG formation catalyzed by UGT1A3 and HLMs was also impaired by BUP and temazepam. Whereas inhibition through temazepam seems rather insignificant, an interaction with BUP is more likely to occur. Additionally, BUP inhibited UGT1A1-mediated conjugation of NBUP, which is stronger than that of temazepam but not as strong as the effect of BUP via UGT1A3. Nearly the same IC_{50} and K_i values were observed for BUP as an inhibitor of NBUPG formation using HLMs.

Conclusion

UGT1A1, 1A3, and 2B7 were involved in BUP glucuronidation, whereas UGT1A1 and 1A3 play a role in NBUP metabolism. There was no preferential conversion of the respective substrate between UGTs.

Although fatalities have been attributed to combinations of high-dose BUP with benzodiazepines, it is unlikely that this occurs by temazepam or oxazepam via inhibition of BUP or NBUP glucuronidation. Inhibition of BUP metabolic clearance by amitriptyline is evident. Additionally, BUP inhibits NBUP glucuronidation and vice versa. A decreased metabolic clearance of NBUP may lead to its accumulation and, hence, may increase the risk of adverse events.

The present data might be useful to evaluate life-threatening intoxications or drug interactions in association with BUP. However, further investigations are needed (1) to evaluate whether and to what extent inhibition of the conjugation of BUP and NBUP plays a role in incidents of BUP overdose or polydrug use and (2) to advance our understanding of the mechanistic interpretation of approaches.

Acknowledgment We acknowledge Dr. R. Garcia Boy for thoroughly reviewing the manuscript. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Sk 48/3-1).

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