Morphine glucuronosyltransferase activity in human liver microsomes is inhibited by a variety of drugs that are co-administered with morphine

Yusuke Hara[1,2], Miki Nakajima[1], Ken-ichi Miyamoto[2], and Tsuyoshi Yokoi[1,*]

[1]Drug Metabolism and Toxicology, Division of Pharmaceutical Sciences, Graduate School of Medical Science, Kanazawa University, [2]Kanazawa University Hospital, Kanazawa, Japan

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*To whom all correspondence should be sent:

Tsuyoshi Yokoi, Ph.D.,

Drug Metabolism and Toxicology

Division of Pharmaceutical Sciences

Graduate School of Medical Science

Kanazawa University

Kakuma-machi

Kanazawa 920-1192, Japan

Tel / Fax +81-76-234-4407

E-mail: TYOKOI@kenroku.kanazawa-u.ac.jp

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Summary:

Morphine is an analgesic drug used for the treatment of acute and chronic pain syndromes for cancer patients. Glucuronidation is a major pathway of the elimination of morphine in humans. Morphine is metabolized to 3-glucuronide (no analgesic effect) and 6-glucuronide (more potently analgesic than morphine) mainly by UGT2B7. In the present study, we investigated the inhibitory effects of a variety of drugs on the morphine glucuronosyltransferase activities in human liver microsomes. Twenty-one drugs including anticancer drugs, immunosuppresants, analgesics, anticonvulsants, antidepressants, antipsychotic drugs were selected in this study, because they are frequently co-administered with morphine. We found that 10 out of 21 drugs, tamoxifen, tacrolimus, diclofenac, carbamazepine, imipramine, clomipramine, amitriptyline, diazepam, lorazepam and oxazepam extensively inhibited the morphine 3- and 6-glucuronosyltransferase activities. Although some of the drugs are not substrates of UGT2B7, they would be potent inhibitors of UGT2B7. If patients receive morphine and these drugs simultaneously, the drug-drug interaction may change the levels of morphine and these glucuronides, resulting in altered analgesic efficacy and the risk of side effects. The results presented here will assist clinicians in choosing the proper drugs and/or dosages, and enable them to anticipate potential drug-drug interactions.

Key words: UDP-glucuronosyltrasnferase; glucuronidation; drug-dug interaction; morphine

Introduction

Morphine is an analgesic drug used for the treatment of acute and chronic pain syndromes. It is used as the most practical and versatile analgesic for the relief of severe pain associated with advanced cancer in palliative care.[1)] Morphine is extensively glucuronidated, and this pathway accounts for approximately two-thirds of the elimination of morphine in humans. Morphine is metabolized to morphine 3- and 6-glucuronides by UDP-glucuronosyltransferases (UGTs) in liver.[2,3)] Morphine 3-glucuronidation is the dominant pathway. The metabolic clearance to morphine 3-glucuronide is about 5-fold higher than the metabolic clearance to morphine 6-glucuronide.[4)] Morphine 3-glucuronide has no analgesic effects, but morphine 6-glucuronide is a more potent (20 times) analgesic than morphine itself.[5)]

Patients suffering from cancer need continuous therapy with morphine. Anti-cancer drugs such as etoposide, irinotecan (its active metabolite is SN-38), and tamoxifen, are widely used for chemotherapy with morphine. Immunosuppressant drugs such as tacrolimus, cyclosporine A, and mycophenolate are sometimes used with morphine for the treatment of pain after organ transplantation. Cancer patients may also receive analgesics (diclofenac, acetaminophen, and naloxone), anticonvulsants (carbamazepine and valproic acid), and antidepressants (imipramine, clomipramine, amitriptyline, and desipramine) for the treatment of neuropathic pain from cancer. In addition, 10 - 30% of cancer patients have psychological distress.[6,7)] For the treatment of the psychological distress, benzodiazepine agonists (diazepam, lorazepam and oxazepam) and antipsychorotic drugs (olanzapine and milnacipran) are used. Thus, morphine is frequently co-administered with a variety of drugs. Since the clearance of morphine is dependent on the metabolism by UGTs, drugs that inhibit UGTs might affect the kinetics of morphine and its glucuronides, resulting in altered analgesic efficacy and the risk of side effects. In the present study, we investigated the inhibitory effects of a variety of drugs that are frequently co-administered with morphine, on morphine glucuronidation in human livers in order to obtain useful information for predicting drug-drug interactions.

Materials and Methods

Chemicals and reagents: Morphine hydrochloride was purchased from Takeda Chemical Industries (Osaka, Japan). Morphine 3- and 6-glucuronides were generous gifts from Dr. Kazuta Oguri, Kyushu University (Fukuoka, Japan). Etoposide, tamoxifen citrate, cyclosporine A, mycophenolate, acetaminophen, valproic acid, carbamazepine, imipramine hydrochloride, clomipramine hydrochloride, amitriptyline hydrochloride, desipramine hydrochloride, diazepam, lorazepam, oxazepam were purchased from Wako Pure Chemical Industries (Osaka, Japan). Diclofenac, naloxone, uridine 5'-diphosphoglucronic acid (UDPGA), and alamethicin were purchased from Sigma-Aldrich (St. Louis, MO). Olanzapine was from Toronto Research Chemicals (Toronto, Canada). Irinotecan and SN-38 were kindly provided by Yakult (Tokyo, Japan). Tacrolimus and milnacipran hydrochloride were kindly provided by Astellas Pharma (Tokyo, Japan) and Asahikasei Pharma (Tokyo, Japan), respectively. Pooled human liver microsomes were obtained from BD Gentest (Woburn, MA). All other chemicals were of the highest grade commercially available.

Morphine glucuronosyltransferase activity: Morphine glucuronosyltransferase activity was determined as described previously [8] with slight modifications. A typical incubation mixture (0.2 ml total volume) contained 50 mM Tris-HCl buffer (pH 7.4), 5 mM $MgCl_2$, 5 mM UDPGA, 25 μg/ml alamethicin, 0.25 mg/ml microsomal protein and 25-200 μM morphine. Each drug, which was dissolved in methanol, was added as an inhibitor so that the final concentration of solvent in the incubation mixture was <1%. The reaction was initiated by the addition of UDPGA. After incubation at 37°C for 30 min, the reaction was terminated by the addition of 0.1 ml of ice-cold perchloric acid. After the removal of protein by centrifugation at 10,000 *g* for 5 min, a 100 μl portion of the supernatant was subjected to HPLC. Chromatography was performed using an L-2130 pump (Hitachi, Tokyo, Japan), an L-2480 FL detector (Hitachi), an L-2200 autosampler (Hitachi), a D-2500 integrator (Hitachi), and an L-2300 column oven (Hitachi). The flow rate was 0.8 ml/min and the column temperature was 35°C. The glucuronides were detected fluorometrically (excitation: 210 nm; emission: 350 nm) with a noise-base clean Uni-3 (Union, Gunma, Japan). The analytical column was a Develosil C_{30} (4.6 x 150 mm; 5 μm) column (Nomura Chemical, Aichi, Japan) and the mobile phase was 50 mM sodium dihydrogen phosphate (pH 4.5). The retention times of morphine 3-glucuronide, morphine 6-glucuronide, and morphine were 18.5, 31.0 and 37.5 min, respectively. The quantification of the metabolites was performed by comparing the HPLC peak heights to those of authentic standards. Limits of detection of morphine 3- and 6-glucuronides were 20 fmol and 200 fmol, respectively. It was also confirmed that intra-day and inter-day precision and accuracy of the detection of the glucuronides were <10% (data not shown). The formation of morphine 3- and 6-glucuronides by human liver microsomes increased linearly with an incubation time up to 60 min and with a protein concentration up to 0.75 mg/ml. Unless specified, an incubation time of 30 min and 0.25 mg/ml microsomal protein were used. All data were analyzed using the mean of duplicate determinations. The variances between the duplicate determinations were < 10%.

Data analyses: Lineweaver-Burk plots were used for the determination of the type of inhibition,[9] and Dixon plots were used as a secondary method. Kinetic parameters were determined by a nonlinear regression analysis using a computer program (K-cat, BioMetallics, Princeton, NJ).

Prediction of in vivo drug-drug interactions from in vitro data: If an enzyme reaction is inhibited competitively or noncompetitively by other drugs, when the substrate concentration is much lower than K_m, the change in the intrinsic clearance (CL_{int}) is expressed by the following equation[10]:

CL_{int} (+inhibitor)/ CL_{int} (-inhibitor) =1/ (1+I/K_i)

where I is the concentration of the inhibitor and K_i is the inhibition constant. When we discuss drug-drug interactions via the inhibition of enzymes, it is important that the concentration of the inhibitor refers to the concentration of the drug around the enzyme. It is difficult to know the actual concentrations of drugs at the active site of UGT. The changes of CL_{int} caused by co-administered drugs can be predicted using the maximum unbound concentrations in the liver. Since data of the concentrations in the liver and protein binding of each drug in tissues are largely not available, the maximum plasma concentrations were alternatively used in the present study.

Results

Inhibitory effects of drugs on morphine glucuronosyltransferase activities in human liver microsomes: Twenty-one drugs (500 μM) were screened for the inhibitory effects on morphine 3- and 6-glucuronosyltransferase activities at a 50 μM substrate concentration. As shown in Fig. 2, the morphine 3- and 6-glucuronosyltransferase activities were strongly inhibited by tamoxifen, diclofenac, imipramine, clomipramine, amitriptyline, desipramine, diazepam, lorazepam and oxazepam (< 20 % of control). The activities were also moderately inhibited by tacrolimus, mycophenolate, naloxone, carbamazepine, and olanzapine (20 - 50 % of control), and weakly inhibited by irinotecan and valproic acid (50 - 70 % of control). Interestingly, the morphine glucuronosyltransferase activities were activated by cyclosporine A (120%). For 14 drugs showing > 50% inhibition at 500 μM, the IC_{50} values were determined by dose response curves with various concentrations. The IC_{50} values are summarized in Fig. 2. The IC_{50} values of each drug were similar between the morphine 3- and 6-glucuronosyltransferase activities.

Inhibition constant and inhibition pattern: We determined the inhibition constant (K_i) values for 14 drugs that inhibited the morphine glucuronosyltransferase activities (IC_{50} values were < 500 μM), (Fig. 3 and Table 1). The K_{is} and K_{ii} values are inhibition constants on the slope (competitive) and on the intercept (noncompetitive), respectively. Tamoxifen, mycophenolate, diclofenac, diazepam, and olanzapine exhibited noncompetitive inhibition for both the morphine 3- and 6-glucuronosyltransferase activities. Tacrolimus, carbamazepine, and lorazepam exhibited a mixed type of competitive and noncompetitive inhibition for both activities. Naloxone, imipramine, clomipramine, amitriptyline, and desipramine exhibited noncompetitive and mixed type inhibitions for morphine 3- and 6-glucuronosyltransferase activities, respectively. Oxazepam exhibited competitive and mixed type inhibitions for morphine 3- and 6-glucuronosyltransferase activities, respectively. All compounds except naloxone and olanzapine more potently inhibited the morphine 6-glucuronosyltransferase activity than the morphine 3-glucuronosyltransferase activity.

Predicted change of in vivo clearance of morphine by a variety of drugs from vitro data: To predict the possibility of drug-drug interaction via a metabolic process between morphine and the 14 drugs, the $1 + I/K_i$ values were calculated (Table 1). Carbamazepine showed the highest $1 + I/K_i$ values (1.4 and 1.9 for morphine 3- and 6-glucuronosyltransferase activities, respectively). The values by diclofenac were both 1.4 for morphine 3- and 6-glucuronosyltransferase activities. Mycophenolate, clomipramine, diazepam and oxazepam showed the $1 + I/K_i$ values of 1.2 for the morphine 6-glucuronosyltransferase activity. Since most compounds more potently inhibit morphine 6-glucuronosyltransferase activity than morphine 3-glucuronosyltransferase activity, the ratio of 3-glucuronide/6-glucuronide in plasma would be increased, changing the analgesic efficacy and the risk of side effects.

Discussion

Morphine is mainly metabolized to 3- and 6-glucuronides by UGT enzymes. Morphine 3-glucuronidation is catalyzed by multiple isoforms such as UGT2B7, UGT1A8, and UGT1A3 with relatively low K_m values (0.4 – 3.2 mM) as well as UGT1A10, UGT1A6, UGT1A1, and UGT1A9 with relatively high K_m values (13 – 37 mM).[3] In contrast, morphine 6-glucuronidation is specifically catalyzed by UGT2B7 (K_m = 1.0 mM).[3] Collectively. UGT2B7 is recognized as a major isoform catalyzing the glucuronidation of morphine. In the presents study, we investigated the inhibitory effects of 21 drugs on the morphine glucuronosyltransferase activities. Although the K_m values of the morphine glucuronosyltransferase activities are high as mM order, the inhibitory effects were investigated with the substrate concentrations at μM order by considering the plasma concentrations in clinical practice [11] and the detection limits of morphine glucuronides in the HPLC system.

Among 21 drugs used in the present study, diclofenac, clomipramine, and amitriptyline have been reported to affect the pharmacokinetics of morphine in vivo. Tighe et al [11] have reported that the plasma concentration of morphine 6-glucuronide was decreased by 40% with co-administration of diclofenac. Diclofenac is known to be mainly metabolized by P450s, but also metabolized by UGTs (Table 2). The affinity of diclofenac to UGT2B7 (K_m = 25 μM) is higher than that of morphine.[12] In the present study, we found that diclofenac potently inhibited the morphine glucuronosyltransferase activities in a noncompetitive manner. The extent of in vivo inhibition can be predicted quantitatively by the calculation of $1/(1+I/K_i)$ from an in vitro study. The $1+I/K_i$ values by diclofenac were 1.4, indicating that the change of the plasma concentration would be due to the inhibition of morphine glucuronidation by diclofenac. Ventafridda et al [13] reported that co-administration of clomipramine and amitriptyline increased the area under the curve (AUC) of morphine approximately 2 fold in humans. Tricyclic antidepressants including clomipramine and amitriptyline are known to be substrates of UGT1A3 and UGT1A4 (Table 2). However, we found that clomipramine (K_i values were 6 - 20 μM) and amitriptyline (K_i values were 30 - 248 μM) strongly inhibited the morphine glucuronidations. These results are in accordance with a previous study by Wahlstrom et al [14] reporting that clomipramine (K_i values were 56 - 90 μM) and amitriptyline (K_i values were 80 - 160 μM) inhibited morphine glucuronidations in human liver microsomes. The $1+I/K_i$ values by clomipramine were at most 1.2, but those by amitriptyline were 1.0, indicating that the prediction of in vivo inhibition from in vitro data might be unsuccessful. For drugs that are cleared predominantly through CYP-mediated metabolism, there is growing evidence that successful prediction of in vivo drug interactions through the inhibition of metabolism can be made from in vitro data. In contrast, drugs that are mainly metabolized by UGT appear to be less well-predicted using in vitro data. This may be due to the nature of UGT, latency restricting the access of substrates or UDPGA and the removal of formed glucuronide. Thus, for drugs that are mainly metabolized by UGT, the calculation of $1+I/K_i$ may not necessarily give a plausible prediction. We found that imipramine and desipramine also prominently inhibited the morphine glucuronosyltransferase activities. Although the $1+I/K_i$ was at most 1.1, it might be necessary to pay attention to the co-administration of these tricyclic antidepressants with morphine.

By calculation of the $1+I/K_i$ value, it was predicted that carbamazepine might cause drug-drug interactions with morphine. Carbamazepine is mainly metabolized by P450s, but also glucuronidated by UGT2B7 (Table 2). [15] The affinity of carbamazepine to UGT2B7 (K_m = 214 μM) is higher than that of morphine. [15] Although the contribution of UGT to carbamazepine metabolism might be low, our data suggest that the co-administration of carbamazepine and morphine should be avoided in clinical practice.

We found that benzodiazepine agonists have inhibitory effects on the morphine glucuronosyltransferase activities. The order of inhibitory potencies was diazepam > lorazepam > oxazepam. The results were consistent with a previous report that these drugs inhibit zidovudine glucuronosyltransferase activity catalyzed by UGT2B7 in human liver microsomes. [16,17] Lorazepam and oxazepam are mainly metabolized by UGT2B7 and 2B15, [18,19] whereas diazepam is mainly metabolized to oxazepam by CYPs.[20] It is clearly demonstrated that these drugs are potent inhibitors of UGT2B7. Co-administration of these benzodiazepine agonists with morphine should also be avoided.

Tamoxifen and tacrolimus strongly inhibited the morphine glucuronosyltransferase activities. Although tamoxifen has been reported to be a substrate of UGT1A4,[21] it is a potent inhibitor of UGT2B7 (K_i values were 27 – 81 μM), like tricyclic antidepressants. Tacrolimus, which is a substrate of UGT2B7, inhibited morphine glucuronosyltransferase activities with a mixed type of competitive and noncompetitive inhibition (K_i values were 46 – 347 μM). A drug-drug interaction between morphine and tamoxifen or tacrolimus might be possible.

Recently, it was reported that ketoconazole, which is a well-known inhibitor of CYP3A4, potently inhibits the morphine glucuronosyltransferase activity catalyzed by recombinant UGT2B7 (K_i values were 110 – 120 μM).[22] The possibility has been suggested that CYPs may interact with UGTs to modulate the function of UGTs.[23] The drugs showing potent inhibitory effects on morphine glucuronidation (Table 1) were substrates of CYPs (clomipramine, amitriptyline, tamoxifen: CYP2D6, diclofenac: CYP2C9, diazepam, lorazepam: CYP3A4). It would be interesting to investigate whether possible interaction between these CYP isoforms and UGT2B7 might be related to the inhibitory effects.

In conclusion, we found that tamoxifen, tacrolimus, diclofenac, carbamazepine, imipramine, clomipramine, amitriptyline, diazepam, lorazepam and oxazepam have prominent inhibitory effects on morphine glucuronidation. If patients receive morphine and these drugs simultaneously, drug-drug interactions may result in changed analgesic efficacy and risk of side effects. Such understanding is important so that clinicians can choose the proper drugs and/or dosages, and anticipate potential drug-drug interactions.

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Table 1. Inhibition of morphine 3- and 6-glucuronosyltransferase activity in human liver microsomes by 14 drugs.

		Morphine 3-glucuronosyltransferase activity				Morphine 6-glucuronosyltransferase activity			
Drug	I (μM)	K_{is} (μM)	K_{ii} (μM)	Inhibitory type	$1 + I/K_i$	K_{is} (μM)	K_{ii} (μM)	Inhibitory type	$1 + I/K_i$
Tamoxifen	0.48		81	Noncompetitive	1.0		27	Noncompetitive	1.0
Tacrolimus	0.04	347	95	Mixed	1.0	101	46	Mixed	1.0
Mycophenolate	60		713	Noncompetitive	1.1		296	Noncompetitive	1.2
Diclofenac	9.4		22	Noncompetitive	1.4		24	Noncompetitive	1.4
Naloxone	0.05		518	Noncompetitive	1.0	1298		Competitive	1.0
Carbamazepine	42	243	118	Mixed	1.4	78	47	Mixed	1.9
Imipramine	1.9		81	Noncompetitive	1.0	60	33	Mixed	1.1
Clomipramine	1.4		20	Noncompetitive	1.1	19	6	Mixed	1.2
Amitriptyline	0.8		248	Noncompetitive	1.0	111	30	Mixed	1.0
Desipramine	1.1		177	Noncompetitive	1.0	458	111	Mixed	1.0
Diazepam	1.8		47	Noncompetitive	1.0		9	Noncompetitive	1.2
Lorazepam	0.2	239	53	Mixed	1.0	65	17	Mixed	1.0
Oxazepam	5	519		Competitive	1.0	41	93	Mixed	1.2
Olanzapine	0.04		196	Noncompetitive	1.0		266	Noncompetitive	1.0

The K_{is} and K_{ii} values are inhibition constants on the slope (competitive) and on the intercept (noncompetitive), respectively.

In the case of mix-type inhibition, the lower K_i was used in the calculation.

The plasma concentration of oxazepam was from Court et al.,[19] and those of the other drugs were from Gilman et al.[24]

Table 2. Drugs used in this study and the involvement of UGTs in their metabolism.

Drug	UGT isoforms	K_m (μM)	excretion as glucuronide
Etoposide	UGT1A1[25]	503	15-30%[26]
Irinotecan	UGT1A1[27]	unknown	unknown
SN-38	UGT1A1[28]	24	14.7%[29]
	UGT1A4	147	
	UGT1A6	97	
	UGT1A9	13	
	UGT2B15	186	
Tamoxifen	UGT1A4[21]	32	unknown
Tacrolimus	UGT2B7[30]	449	unknown
Cyclosporine	UGT2B7[31]	202	unknown
Mycophenolate	UGT1A1[8]	185	96%[32]
	UGT1A7	30	
	UGT1A8	298	
	UGT1A9	291	
	UGT1A10	119	
Diclofenac	UGT1A3[12]	12	5-10%[33]
	UGT2B7	25	
Acetaminophen	UGT1A1[34]	9400	63%[35]
	UGT1A6	2200	
	UGT1A9	20900	
Naloxone	UGT1A3[36]	unknown	unknown
	UGT2B7	unknown	
Carbamazepine	UGT2B7[15]	214	15%[37]
Valproic acid	UGT1A6[38]	3200	10%[39]
	UGT1A9	5200	
	UGT2B7	2100	
Imipramine	UGT1A3[40]	472	0.1-0.8%[41]
	UGT1A4	310	
Clomipramine	UGT1A3[40]	unknown	0.1-0.8%[41]
	UGT1A4	unknown	
Amitriptyline	UGT1A3[40]	267	26%[42]
	UGT1A4	170	
Desipramine	UGT1A3[40]	unknown	0.1-0.8 %[41]
	UGT1A4	unknown	
Diazepam	unknown	unknown	unknown
Lorazepam	UGT2B7[18]	unknown	86%[43]
	UGT2B15[19]	unknown	
Oxazepam	UGT2B7[18]	203	67%[45]
	UGT2B15[44]	32	
Olanzapine	UGT1A4[46]	227	25%[47]
Milnacipran	unknown	unknown	30%[48]

Figure legends

Fig. 1. Structure of drugs used in the present study.

Fig. 2. Inhibitory effects of drugs on morphine glucuronosyltransferase activities in human liver microsomes. The concentrations of morphine and each drug were 50 μM and 500 μM, respectively. Each column represents the mean of duplicate determinations. The control activities in the pooled human liver microsomes were 23.1 pmol/min/mg protein for morphine 3-glucuronosyltransferase activity and 5.4 pmol/min/mg protein for morphine 6-glucuronosyltransferase activity. N.D., not detected.

Fig. 3. Typical Lineweaver-Burk plots of morphine glucuronosyltransferase activities in human liver microsomes. Effects of oxazepam (A, B) or naloxone (C, D) on morphine 3- (A, C) and 6- (B, D) glucuronosyltransferase activities were investigated. Each data point represents the mean of duplicate determinations. Lines were drawn by linear regression analysis.