Short Communication

Effects of Ketamine on Human UDP-Glucuronosyltransferases In Vitro Predict Potential Drug-Drug Interactions Arising from Ketamine Inhibition of Codeine and Morphine Glucuronidation

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ABSTRACT:

In this study, the selectivity of UDP-glucuronosyltransferase (UGT) enzyme inhibition by ketamine (KTM) and the kinetics of KTM inhibition of human liver microsomal morphine (MOR) and codeine (COD) glucuronidation were characterized to explore a pharmacokinetic basis for the KTM-opioid interaction. With the exception of UGT1A4, KTM inhibited the activities of recombinant human UGT enzymes in a concentration-dependent manner. However, IC_{50} values were <100 μM only for UGT2B4, UGT2B7, and UGT2B15. UGT2B7 catalyzes MOR 3- and 6-glucuronidation and the 6-glucuronidation of COD, with an additional substantial contribution of UGT2B4 to the latter reaction. Consistent with the effects of KTM on the activities of recombinant UGT2B enzyme activities, KTM competitively inhibited human liver microsomal MOR and COD glucuronidation. K_{i} values for KTM inhibition of MOR 3- and 6-glucuronidation and COD 6-glucuronidation by human liver microsomes supplemented with 2% bovine serum albumin were 5.8 ± 0.1, 4.6 ± 0.2, and 3.5 ± 0.1 μM, respectively. Based on the derived inhibitor constants, in vitro-in vivo extrapolation was used to predict the effects of anesthetic and analgesic doses of KTM on MOR and COD clearances. Potentially clinically significant interactions (>50% increases in the in vivo area under the curve ratios) with MOR and COD were predicted for anesthetic doses of KTM and for a subanesthetic dose of KTM on COD glucuronidation.

Introduction

The N-methyl-d-aspartate receptor antagonist ketamine (KTM) has been used clinically as a dissociative anesthetic for more than four decades. However, KTM also exerts analgesic effects. In particular, there is evidence supporting the use of KTM as an adjuvant analgesic in several chronic pain states. KTM has been reported to provide improved pain relief in cancer patients who have a suboptimal analgesic response to high-dose morphine (MOR), with a concomitant reduction in opioid dose requirement and adverse effects (Bell 1999; Fitzgibbon and Viola, 2005). Although the improvement in MOR response due to KTM is generally believed to arise from attenuation of opioid tolerance and opioid-induced pain sensitivity after N-methyl-d-aspartate receptor blockade, it has been demonstrated recently that KTM inhibits the clearance of MOR via 3-glucuronidation in the isolated perfused rat liver preparation (Qi et al., 2010). Furthermore, KTM inhibited MOR 3-glucuronidation by rat liver microsomes.

As in the rat, MOR undergoes extensive hepatic glucuronidation in humans. Elimination by 3- and 6-glucuronidation comprise 57 and 10% of MOR systemic clearance, respectively (Hasselström and Säwe, 1993). UGT2B7 is the enzyme primarily responsible for hepatic MOR 3- and 6-glucuronidation (Stone et al., 2003). Like MOR, codeine (COD) is extensively glucuronidated in humans, with approximately 80% of the dose excreted in urine as codeine-6-beta-d-glucuronide (C6G) (Yue et al., 1991). UGT2B7 also glucuronidates COD, although there is an additional substantial contribution of UGT2B4 to C6G formation (Raungrut et al., 2010).

To further explore a pharmacokinetic basis for the potentiation of opioid analgesia by KTM, we characterized the selectivity of UDP-glucuronosyltransferase (UGT) enzyme inhibition by KTM and the kinetics of KTM inhibition of human liver microsomal MOR and COD glucuronidation. We conducted inhibition studies with human liver microsomes (HLM) as the enzyme source in the presence and absence of bovine serum albumin (BSA; 2%). BSA sequesters long-chain unsaturated fatty acids released from the micromolar membrane during the course of an incubation, which act as potent inhibitors of UGT2B4 and UGT2B7 (Rowland et al., 2007, 2008; Raungrut et al., 2010). Thus, K_{i} values generated in the presence of BSA provide a more accurate prediction of drug-drug interaction potential in vivo (Rowland et al., 2006; Uchaipichat et al., 2006; Raungrut et al., 2010). Based on the derived inhibitor constants, in vitro-in vivo extrapolation (IV-IVE) was used to predict the likelihood of clinically significant interactions between KTM and MOR and COD.
Ketamine Inhibition of Human Liver Microsomal Morphine and Codeine Glucuronidation. Inhibition of human liver microsomal (pooled) C6G, M3G, and M6G formation was determined at four KTM concentrations (see Fig. 2 for concentrations) at each of three COD or MOR concentrations (see Fig. 2) in the presence and absence of BSA (2% w/v), to determine mechanism of inhibition and \( K_i \) values. COD and MOR concentrations spanned the \( K_m \) values for each substrate (Raungrut et al., 2010; N. Chau and J. O. Miners, unpublished results).

Non-specific Binding of Ketamine to Human Liver Microsomes. The binding of KTM to HLM and to HLM plus 2% BSA was characterized by equilibrium dialysis according to the general procedure of McLure et al. (2000). One side of dialysis cell contained KTM in phosphate buffer (0.1 M, pH 7.4), whereas the other side contained a suspension of either pooled HLM (1 mg/ml) or a combination of 2% BSA and HLM (1 mg/ml). We investigated KTM binding over the concentration ranges of 10 to 250 and 2 to 50 \( \mu M \) for samples containing HLM and HLM plus 2% BSA, respectively. After dialysis at 37°C for 4 h, a 200-\( \mu l \) aliquot was collected from each cell and treated with 500 \( \mu l \) of ice-cold methanol containing 4% glacial acid. Samples were chilled on ice for 20 min and subsequently centrifuged at 13,000g for 5 min at 4°C. An aliquot of the supernatant fraction was analyzed by HPLC. HPLC was performed using an Agilent 1100 series instrument (Agilent Technologies) fitted with a NovaPak C18 column (3.9 \( \times \) 150 mm, 5-\( \mu m \) particle size; Waters). Mobile phase, delivered at flow rate 1 ml/min, comprised a 1:1 mixture of 30 mM phosphate buffer (pH 7.2) and acetonitrile. Column eluant was monitored by fluorescence detection at excitation and emission wavelengths of 235 and 435 nm, respectively, in order to fit experimental data. Goodness of fit was assessed from comparison of the F statistic, \( r^2 \) values, S.E. of the parameter fit, and 95% confidence intervals. \( K_i \) values are reported as the parameter \( \pm \) S.E. of the parameter estimate.

**IV-IVE.** The predicted magnitude of the inhibition of MOR and COD hepatic clearance by KTM was calculated as the predicted ratios of the areas under the plasma concentration-time curves with (AUC\(_i\)) and without (AUC) KTM coadministration.

\[
\frac{\text{AUC}}{\text{AUC}_{\text{IV-IVE}}} = \frac{1}{1 + (f[I]/K_i) + (1 - f_o)}
\]  

where \( f[I] \) is the inhibitor concentration, \( f_o \) is the fraction of victim drug (COD or MOR) cleared along each pathway, and \( K_i \) is the inhibition constant generated in vitro. COD fractional clearance via 6-glucuronidation was taken as 80% (Yue et al., 1991), and the fractions of MOR 3- and 6-glucuronidation were 0.57 and 0.10, respectively (Hasselström and Säwe, 1993). The inhibitor concentration ([\( I \)]) in vivo was taken as either the total or unbound concentrations of KTM in plasma after anesthetic and analgesic doses (see Results and Discussion). The mean unbound fraction of KTM in the plasma of healthy subjects has been reported as 0.73 (Dayton et al., 1983).

Results and Discussion

The binding of KTM to HLM alone was negligible across the concentration range investigated (\( f_{\text{unc}} = 0.98 \pm 0.02 \)). However, binding of KTM to HLM plus 2% BSA was 21% (\( f_{\text{unc}} = 0.79 \pm 0.02 \)), which was independent of KTM concentration. The concentration of KTM added to incubations containing BSA was corrected for \( f_{\text{unc}} \) when calculating \( K_i \) values from inhibition studies. Previous results from this laboratory have demonstrated that COD and
MOR do not bind significantly to HLM in the absence and presence of 2% BSA (Raungrut et al., 2010; N. Chau and J. O. Miners, unpublished data).

KTM inhibition of recombinant human UGT activities was assessed using 4-MU (UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A9, 1A10, 2B7, 2B15, and 2B17), LTG (UGT1A4), and COD (UGT2B4) as the probe substrates. With the exception of UGT1A4, KTM inhibited all UGT enzymes in a concentration-dependent manner (Fig. 1). However, the greatest inhibition was observed with UGT2B4, 2B7, and 2B15 as the enzyme sources; respective estimated IC_{50} values were 69, 55, and 95 μM, whereas the IC_{50} values obtained for the other enzymes were an order of magnitude higher. As indicated under Materials and Methods, inhibition studies with the UGT2B enzymes were conducted with Supersomes as the enzyme source, whereas UGT1A enzymes were expressed in HEK293 cells. To exclude expression system-dependent effects of KTM, inhibition of UGT2B enzymes expressed in HEK293 cells (Uchaipichat et al., 2004) was also tested. Similar inhibition of UGT2B7 and UGT2B15 was observed (data not shown), although the low activity of UGT2B4 in HEK293 cell lysate precluded meaningful interpretation of inhibition data.

As noted under Introduction, UGT2B7 catalyzes COD and MOR glucuronidation whereas UGT2B4 additionally contributes to C6G formation. On the basis of the data shown in Fig. 1 and the previous report of KTM inhibition of MOR 3-glucuronidation in the rat (Qi et al., 2010), KTM inhibition of human liver microsomal MOR 3- and 6-glucuronidation and COD 6-glucuronidation was characterized kinetically. Effects of KTM on each of the three glucuronidation pathways were modeled well using the equation for competitive inhibition (Fig. 2). K_{i} values determined for MOR 3- and 6-glucuronidation and COD glucuronidation in the absence of BSA were 40 ± 0.7, 35 ± 0.7, and 52 ± 0.8 μM, respectively. Addition of BSA (2%) to incubations resulted in 85 to 93% reductions in K_{i} values; 5.8 ± 0.1, 4.6 ± 0.2, and 3.5 ± 0.1 μM for KTM inhibition of MOR 3- and 6-glucuronidation and COD 6-glucuronidation, respectively. As noted previously, we accounted for KTM binding to HLM plus 2% BSA in the calculation of K_{i} values.

IV-IVE approaches have been applied successfully to predict in vivo clearance and inhibitory drug-drug interaction potential for compounds eliminated by glucuronidation (Miners et al., 2004, 2006, 2010). In particular, IV-IVE predicted the magnitude of the flucona-
KTM concentrations were used for IV-IVE. Previous studies of drug-drug interaction potential have generally reported optimal prediction of the AUC ratio using total drug concentration (e.g., Ito et al., 2004; Rowland et al., 2006).

In summary, data presented here demonstrate that KTM inhibits human UGT2B4, UGT2B7, and UGT2B15. Consistent with the known involvement of UGT2B4 and UGT2B7 in MOR and COD metabolism, KTM inhibited the glucuronidation of these compounds by HLM. $K_i$ values generated in the presence of BSA predicted potential inhibition of opioid clearance after anesthetic and possibly subanesthetic doses of KTM, supporting the hypothesis that a pharmacokinetic mechanism may contribute to KTM-opioid interactions. Furthermore, KTM may potentially precipitate interactions with other compounds because UGT2B7 contributes to the metabolism of numerous other drugs, including anticancer agents and nonsteroidal anti-inflammatory drugs, and endogenous compounds such as hydroxy-steroids (Jin et al., 1997; Kiang et al., 2005; Miners et al., 2010).

**Fig. 2.** Dixon plots for ketamine inhibition of COD 6-glucuronidation and MOR 3- and 6-glucuronidation by pooled HLM in the absence (A, C, E) and presence (B, D, F) of BSA (2%). Each point represents the mean of duplicate estimates whereas lines are from model fitting.
TABLE 1

Predicted fold increase in the AUCs of codeine and morphine based on plasma ketamine concentrations reported following anesthetic and analgesic doses

<table>
<thead>
<tr>
<th>Reference</th>
<th>KTM Dose</th>
<th>Total Plasma [KTM] (μM)</th>
<th>Predicted Fold Increases in Codeine and Morphine AUC Ratios Based on Reported Total and Unbound In Vivo Ketamine Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idvall et al. (1979)</td>
<td>2 mg + 41 μg · min⁻¹ · kg⁻¹ i.v.</td>
<td>9.3</td>
<td>Codeine ([KTM]total) Codeine ([KTM]unbound) Morphine [a] ([KTM]total) Morphine [a] ([KTM]unbound)</td>
</tr>
<tr>
<td>Xie et al. (2003)</td>
<td>0.5 mg/kg epidurally</td>
<td>2.7</td>
<td>2.39 2.12 1.61 1.50</td>
</tr>
<tr>
<td>Clements and Nimmo (1981)</td>
<td>0.125–0.25 mg/kg i.v.</td>
<td>0.42</td>
<td>1.54 1.41 1.26 1.20</td>
</tr>
</tbody>
</table>

[a] Unbound ketamine concentration calculated as fu (% total plasma concentration.
[b] Morphine AUC ratios represent the sum of the separate ratios for the 3- and 6-glucuronidation pathways.
[c] Pain threshold increased >5 min when total plasma ketamine concentration was >100 μg/L.

References


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