Short Communication

Inhibition of Morphine Metabolism by Ketamine

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ABSTRACT:
Clinical observation of a synergistic effect of ketamine on morphine analgesia remains controversial. Although a pharmacodynamic basis for an interaction has been explored in animal and clinical studies, the possibility of a pharmacokinetic mechanism has not been investigated. Whereas both morphine and morphine-6-glucuronide are effective analgesics, morphine-3-glucuronide (M3G) lacks activity. Thus, changes in the metabolism and disposition of morphine may result in an altered response. First, we investigated the interaction between morphine and ketamine in the isolated perfused rat liver preparation. The clearance of morphine was decreased from 16.8 ± 4.6 ml/min in the control period to 7.7 ± 2.8 ml/min in the ketamine-treatment period, with the formation clearance of M3G decreasing from 8.0 ± 4.1 ml/min to 2.1 ± 1.1 ml/min. Fractional conversion of morphine to M3G was significantly decreased from 0.46 ± 0.17 in the control period to 0.28 ± 0.14 upon the addition of ketamine. The possible mechanism of the interaction was further investigated in vitro with rat liver microsomes as the enzyme source. The formation of M3G followed single-enzyme Michaelis-Menten kinetics, with a mean apparent Km of 2.18 ± 0.45 mM and Vmax of 8.67 ± 0.59 nmol/min/mg. Ketamine inhibited morphine 3-glucuronidation noncompetitively, with a mean Ki value of 33.3 ± 7.9 μM. The results demonstrate that ketamine inhibits the glucuronidation of morphine in a rat model.

Ketamine [2-((O-chlorophenyl)-2-((methylamino)cyclohexanone)] is a noncompetitive N-methyl-D-aspartate receptor antagonist that is used as both an anesthetic and analgesic agent (Fratta et al., 1980). In recent years, there have been conflicting clinical reports on whether ketamine enhances the analgesic effects of morphine. Several researchers indicated that concurrent use of morphine and ketamine prolonged the duration of morphine-induced analgesia and reduced the consumption and adverse effects of morphine in patients (Bell, 1999; Kannan et al., 2002; Galinski et al., 2007), whereas others reported no additional value when the two were combined for postoperative analgesia (Sakata et al., 2007; Jensen et al., 2008).

The μ-opioid receptor antagonist naloxone (10 mg/kg) reversed ketamine-induced analgesia in rodents (Ryder et al., 1978), similar to its effect on morphine analgesia. However, Fratta et al. (1980) indicated that ketamine did not interact with opioid receptors. Animal studies that were used to investigate the interaction between morphine and ketamine suggested that a part of the analgesic action of ketamine was achieved through a mechanism similar to that of morphine (Smith et al., 1987). Discussions regarding the mechanism of the morphine-ketamine interaction have focused on either pharmacodynamic effects or clinical observations. However, a potential pharmacokinetic mechanism for the interaction has not been explored.

Morphine undergoes extensive hepatic extraction and metabolism. More than 50% of an oral dose given to humans and rodents is excreted in urine as morphine-3-glucuronide (M3G). Approximately 10% is excreted as morphine-6-glucuronide (M6G) in man and in several other species, but not in rats (Milne et al., 1996). M6G is a potent μ-receptor agonist, whereas M3G may antagonize the antinociceptive action of morphine and M6G (Milne et al., 1996). Thus, it is possible that altered morphine clearance and formation of morphine glucuronides may influence the pharmacological effect of morphine.

Pharmacokinetic interactions may be examined in vivo and in vitro, the latter using isolated organs and microsomal or cellular preparations. Isolated perfused livers (IPL) allow evaluation of dispositional and metabolic interactions, whereas rat liver microsomal preparations can be used to test for the latter based on interactions at the enzymatic level. The aim of this study was to investigate the impact of ketamine on the metabolism and disposition of morphine by using the isolated perfused rat liver and rat hepatic microsomes.

Materials and Methods

Chemicals. Morphine (morphine HCl · 3H2O; McFarlane Smith, Edinburgh, UK), ketamine (Parnell Laboratories Pty Ltd., Alexandria, NSW, Australia), bovine serum albumin (ICN Biomedicals Inc., Aurora, OH), and uridine 5′-diphosphoglucuronic acid (UDPGA), alamethicin, sodium taurocholate, and M3G (all obtained from Sigma-Aldrich, St. Louis, MO) were used as supplied. Other chemicals were of analytical grade and were purchased commercially.

Isolated Perfused Liver Preparation. The study was approved by the Institute of Medical and Veterinary Science (IMVS) Animal Ethics Committee (Adelaide, Australia). Five male Sprague-Dawley rat (310–360 g; IMVS, Adelaide, Australia) livers were isolated and perfused in a single-pass mode (Milne et al., 1997). Anesthesia was induced with 60 mg/kg sodium pentobarbital. Drug-free Krebs-bicarbonate buffer (pH 7.4) that contained 16.5 mM glucose and 8.33 μM sodium taurocholate was pumped (30 ml/min) into the liver via the portal vein. After equilibration for 20 min, medium that contained 6.7 μM morphine was perfused (0–30 min), and the perfusate (1 ml) was collected from the cannulated vena cava at 0, 1, 2, 5, 10, 15, 20, 25, and 30 min. The medium was then changed to one that contained both 6.7 μM morphine and 21 μM ketamine (30–70 min). Perfusate was collected at 31, 32, and 35 min and every 5 min thereafter up to 70 min. All bile was collected at 5-min intervals. In addition, two rat livers were perfused with medium con-
taining 6.7 μM morphine for 70 min after a 20-min period of equilibration. Liver viability was monitored by assessing its gross appearance (uniformly perfused), bile flow (>3 μl/min), and the recovery of venous perfusate (>98% of the volume of inflowing perfusing medium).

The perfuse and bile (diluted 1 in 1000 with water) samples were analyzed by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) with fluorescence detection (excitation at 280 nm, emission at 335 nm). Samples were directly injected onto a C18 column (250 × 4.6 mm; Alltech Associates, Inc., Deerfield, IL), with a C18 guard column (7.5 × 3.2 mm; Grace, Baulkham Hills, NSW, Australia), in an oven set at 45°C. A mobile phase consisting of 2.5% acetonitrile and 97.5% phosphate buffer (20 mM) was used at a flow rate of 1 ml/min. Under these conditions, the retention times for M3G and morphine were 5.9 and 17.3 min, respectively. Calibration curves were linear (\( r^2 = 0.996) for morphine (0.005–2.6 μM) and M3G (0.11–4.88 μM) in perfuse and bile. Accuracy and precision for measuring quality controls that spanned the range for the calibration standards were within 20%.

Measurement of Microsomal M3G Formation. Microsomes were prepared as described by Lear et al. (1991) and stored at −70°C until use. They were preincubated with alamethicin (50 μg/mg protein) on ice for 30 min. Duplicate incubations consisting of microsomes (1 mg protein/ml), 5 mM MgCl₂, morphine, 0.1 mM Tris-HCl (pH 7.4), and 5 mM UDPGA in a total volume of 0.2 ml were performed at 37°C for 45 min. The reaction rate was linear with incubation time up to 60 min and protein concentration up to 2 mg/ml. The reaction was terminated by adding 0.2 ml of ice-cold HClO₄ (0.1 M) and cooling on ice. Samples were centrifuged at 10,000 g for 10 min. Formed M3G was quantified by using the same high-performance liquid chromatography system with a UV detector set at 210 nm. Supernatant was within 8%.

Measurement of M3G Formation. In vitro kinetic constants are expressed as mean ± S.D. Initial estimates of apparent \( K_m \) and \( V_{max} \) for M3G formation were obtained from graphical analysis of Eadie-Hofstee plots. The single-enzyme Michaelis-Menten equation was fitted to untransformed data by using Prism 5 (GraphPad Software Inc., San Diego, CA). \( K_m \) values were similarly obtained by fitting with the expression for noncompetitive inhibition.

Results and Discussion

The studies presented here provide compelling evidence that ketamine inhibits the conversion of morphine to M3G in rat liver. Morphine and M3G appeared rapidly in the outflowing perfuse and attained steady-state within 10 min of commencing perfusion with morphine alone. Upon addition of ketamine, the concentrations of M3G in the outflowing perfuse decreased markedly, whereas those for morphine increased (Fig. 1), and an additional 10 to 15 min was required before a new steady-state for M3G and morphine was reached in the outflowing perfuse. In contrast, the concentrations of morphine and M3G in the outflowing perfuse remained constant beyond the 20 min during perfusion of the two livers with morphine alone for the entire 70 min (Fig. 1). M3G was excreted extensively in bile. The rate of M3G excretion was constant after 15 min, but it decreased by approximately 75% after the addition of ketamine. Mean pharmacokinetic parameters during the control and ketamine-treatment periods are summarized in Table 1. Ketamine decreased the clearance of morphine by 54% (\( p < 0.05 \)). Hepatic cellular uptake of morphine is partly achieved by an active carrier-mediated system, but with passive diffusion playing an important role after the saturation of

\[
\frac{C_{in} - C_{mut}}{C_{in} \times Q} = F
\]

where \( C_{in} \) and \( C_{mut} \) are the concentrations of morphine in inflowing perfusing medium and outflowing perfuse, respectively.

\[
\text{Formation clearance of M3G (CL}_{M3G} = \frac{R_{perf} + R_{bil}}{C_{in}}
\]

where \( R_{perf} \) and \( R_{bil} \) are the rates of recovery of M3G in perfuse and bile, respectively.

\[
\text{Fractional conversion of morphine to M3G (FC}_{M \rightarrow M3G} = \text{CL}_{M3G}/\text{CL}_{M}
\]

Pharmacokinetic parameters are presented as mean ± S.D. Comparisons between periods were made by paired Student’s \( t \) test, and \( p < 0.05 \) was considered significant.

\[
F = \frac{C_{in}}{C_{mut}}\frac{C_{un}}{C_{mut}}
\]

where \( C_{in} \) and \( C_{mut} \) are the concentrations of morphine in inflowing perfusing medium and outflowing perfuse, respectively.

\[
\text{Hepatic clearance of morphine (CL}_{H\text{-M}} = (C_{in} - C_{mut}) \times Q
\]

where \( Q \) is the flow rate of perfusing medium.

\[
\text{Hepatic clearance of morphine (CL}_{H\text{-M}} = \frac{C_{in}}{C_{in} \times Q}
\]

where \( Q \) is the flow rate of perfusing medium.

\[
\text{Forma}
transporters beyond 200 μM morphine (Iwamoto et al., 1978). Liver was perfused here with 6.7 μM morphine. Thus, the reduction in hepatic clearance of morphine may result from inhibition by ketamine of the active uptake of morphine or from a reduction in the biotransformation of morphine. The permeability of M3G through the lipophilic environment of cellular membrane is apparently limited by its polarity. The uptake of M3G from sinusoids to the hepatocytes is negligible compared with the flux of heptically formed M3G in the opposite direction. The greater efflux is driven by its accumulation within liver cells after formation from morphine (Evans et al., 1999). Efflux of M3G across the sinusoidal membrane is mediated by multidrug resistance protein (Mrp) 3, whereas efflux across the canicular membrane is mediated by Mrp2 (van de Wetering et al., 2007). M3G was excreted extensively into bile, but no morphine was detected. The unchanged biliary extraction ratio of M3G suggests that ketamine does not affect M3G efflux, or Mrp2 and Mrp3 are both inhibited to a similar extent.

The fractional conversion of morphine to M3G was significantly impaired in the presence of ketamine (0.46 versus 0.28, p < 0.05) (Table 1). If ketamine simply blocked hepatic uptake of morphine, the fractional conversion of morphine should remain unchanged. Therefore, it would seem that inhibition of uptake is unlikely to be the only mechanism for the interaction between morphine and ketamine. In addition, the reduced fractional conversion of morphine to M3G suggests that biotransformation of morphine to M3G may be reduced to a greater extent than other metabolic pathways, such as the formation of normorphine (Blanck et al., 1990). To investigate the interaction between morphine and ketamine further, additional experiments examined the impact of ketamine on the formation of M3G by rat liver microsomes.

Glucuronidation, catalyzed by UDP-glucuronosyltransferases (UGT), is the most important conjugation reaction in drug biotransformation (Miners and Mackenzie, 1991). Morphine is extensively metabolized in the liver to one or more glucuronides, depending on the species (Milne et al., 1996), but M3G is the only glucuronide formed from natural (−)-morphine by rat microsomal UGTs (Rane et al., 1985). At least two forms of rat liver UGT are involved in the formation of M3G (Ishii et al., 1997). UGT2B7 is the major human UGT that catalyzes the formation of morphine glucuronides (Stone et al., 2003), but recombinant human UGT1A1 and 1A8 are also known to form M3G (Ohno et al., 2008). However, their activities are low compared with UGT2B7, and UGT1A1 is not expressed in the liver. Figure 2a shows that the representative Eadie-Hofstee plot for the formation of M3G by activated rat liver microsomes. M3G formation was well described by the single-enzyme Michaelis-Menten equation, with a mean apparent \( K_m \) of 2.18 ± 0.45 mM and \( V_{max} \) of 8.67 ± 0.59 nmol/min/mg.

Figure 2b shows that ketamine inhibited the formation of M3G in a noncompetitive manner, with a mean apparent \( K_i \) of 33.3 ± 7.9 μM. This result was an unexpected finding given that there was no previous evidence that ketamine was capable of inhibiting drug glucuronidation. Ketamine is metabolized primarily via N-demethylation to norketamine by cytochrome P450 (White and Ryan, 1996). The data suggest that ketamine may occupy a site on the UGT(s), which causes a reduction in the formation of M3G. Overall, it can be concluded that the inhibitory effect of ketamine on the formation of M3G by UGT accounted for the interaction observed between these two compounds in the IPL.

Figure 2. a, representative Eadie-Hofstee plot for M3G formation by rat liver microsomes. b, representative Dixon plot showing the inhibitory effect of ketamine on the rate of formation of M3G from morphine. The number beside each line shows the concentration of morphine (mM).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Period</th>
<th>Ketamine-Treatment Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F )</td>
<td>0.44 ± 0.14</td>
<td>0.74 ± 0.09*</td>
</tr>
<tr>
<td>( CL_{H,M} ) (ml/min)</td>
<td>16.8 ± 4.6</td>
<td>7.7 ± 2.8*</td>
</tr>
<tr>
<td>( CL_{H} ) (ml/min)</td>
<td>8.0 ± 4.1</td>
<td>2.1 ± 1.1*</td>
</tr>
<tr>
<td>( FC^{*}_{M&gt;M3G} )</td>
<td>0.46 ± 0.17</td>
<td>0.28 ± 0.14*</td>
</tr>
<tr>
<td>( E_{M,M3G} )</td>
<td>0.76 ± 0.16</td>
<td>0.68 ± 0.20</td>
</tr>
</tbody>
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* \( p < 0.05 \) compared to the corresponding value in the control period.

Projean et al. (2003) examined the pharmacokinetics of morphine in rats after intravenous administration of 10 μmol/kg and found that the concentrations of morphine were maintained above 2 μM for up to 1 h. Another study investigated the analgesic effect of morphine in rats and showed that 3.3 μmol/kg morphine (s.c.) provided a delay in the tail-flick reaction time for approximately 2.5 h (Stain et al., 1995). In the IPL, the inflow concentration of morphine in the perfusion medium was 6.7 μM in the control period, which is of the same magnitude as the concentration providing analgesia in rats. A biodisposition study of ketamine in rats showed that the concentration of ketamine was approximately 105 and 16.8 μM in liver and plasma 20 min after an intravenous dose of 12.6 μmol/kg. With this dosage, the analgesia from ketamine can be maintained for up to 30 min (Marietta et al., 1976). The concentration of ketamine perfusing the liver in our study was similar to the concentration required to achieve analgesia in rats.

Ketamine in perfusate at a concentration of approximately three times that of morphine caused a 50% reduction in its hepatic clear-
In contrast, the concentration of ketamine used in the microsomal study was approximately 4% of the concentration of morphine. The differences in inhibitory capacity in these two experimental systems might be due to hepatic distributional barriers for ketamine and its extensive metabolism and removal in perfused liver, non-specific binding of ketamine in microsomes, and/or the inhibitory effects of endogenous inhibitors of glucuronidation present in microsomal incubations (Miners et al., 2010).

In conclusion, this study has shown that ketamine reduces the hepatic clearance of morphine, most likely due to inhibition of the hepatic UGT(s) that catalyze M3G formation. Because M3G and M6G have different pharmacological activities, it is possible that the controversial observation in patients could be partly explained by the change in ratio of M3G and/or M6G to morphine in the body. However, other inhibitory mechanisms of ketamine that cause the interaction with morphine cannot be ruled out. Whether ketamine is capable of inhibiting the glucuronidation of other drugs when the two drugs are coadministered in vivo warrants investigation.

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References


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