JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 593–597

Received October 01, 2008
Accepted February 04, 2009
DOI 10.1211/jpp/61.05.0007
ISSN 0022-3573

Research Paper

Effects of quinidine on antinociception and pharmacokinetics of morphine in rats

Takashi Okura*, Yuki Morita, Yoshihiko Ito, Yoshiyuki Kagawa and Shizuo Yamada

Department of Pharmacokinetics, Pharmacodynamics, Global Center of Excellence (COE) and Clinical Pharmaceutics, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Abstract

Aim The aim of this study was to investigate the effect of quinidine, a P-glycoprotein inhibitor, on the pharmacokinetics and pharmacodynamics of morphine in rats.

Methods Rats were given morphine (30 mg/kg p.o. or 30 mg/kg over 10 min i.v.) 30 min after pretreatment with quinidine (30 mg/kg p.o.). Antinociceptive effects were determined using the tail immersion test. Concentrations of morphine in plasma and brain were also determined.

Key findings The antinociception of morphine was significantly enhanced by oral administration of quinidine, with a 3.1-fold greater area under the effect–time curve than that in vehicle-treated rats. Morphine concentrations in plasma and brain were significantly increased by quinidine. The area under the plasma concentration–time curve after oral or intravenous administration of morphine was increased 5.2- and 1.7-fold, respectively, in quinidine-pretreated rats compared with vehicle-pretreated rats. Quinidine caused a 40% decrease in the total clearance of morphine and increased the concentration of morphine in the brain, although the brain-to-plasma concentration ratio was not changed.

Conclusions Oral administration of quinidine increases the absorption of morphine from the gastrointestinal tract and subsequently enhances the concentration in the brain and its antinociceptive effect. Enhanced intestinal absorption of morphine may be due largely to inhibition of intestinal P-glycoprotein by quinidine.

Keywords absorption; drug interaction; morphine; P-glycoprotein; quinidine

Introduction

Chronic pain is highly prevalent among patients with cancer. Morphine is an opioid analgesic frequently used to control moderate-to-severe pain in these patients.[1] Oral administration is the most common route of administration, and several oral dosage forms of morphine are available. The bioavailability of morphine (24% for an oral solution; 22% for a controlled-release tablet[2]) is lower than that of oxycodone (60%),[3] even though the lipophilicity of these opioids is similar.[4] Morphine is a substrate of efflux transporters such as P-glycoprotein, which is expressed in several tissues, including brain capillary endothelia and intestinal epithelia, which can therefore affect its pharmacokinetics. The concentration of morphine in the brain and its antinociceptive effects were enhanced by knockout of the P-glycoprotein gene in mice and by administration of P-glycoprotein inhibitors in rats,[5–8] whereas the pharmacokinetics of oxycodone were not affected by a P-glycoprotein inhibitor.[9] Thus, P-glycoprotein-mediated efflux may limit the bioavailability of morphine.

In a clinical study, the plasma concentration of oral morphine was increased by the prior administration of quinidine, a P-glycoprotein inhibitor.[10] This finding led us to assume that the inhibition of intestinal P-glycoprotein by quinidine may increase the intestinal absorption of morphine and thereby enhance its therapeutic effect.

P-glycoprotein inhibitors such as quinidine may modulate the pharmacokinetics and pharmacodynamics of morphine. However, the effects of quinidine on the brain distribution and antinociceptive effect of morphine remain to be elucidated. We therefore examined the effect of quinidine pretreatment on the antinociceptive effects of morphine and its pharmacokinetics, including intestinal absorption and distribution in the brain.

Correspondence: Shizuo Yamada, Department of Pharmacokinetics and Pharmacodynamics, Global Center of Excellence (COE) and Clinical Pharmaceutics, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan. E-mail: yamada@ys7.u-shizuoka-ken.ac.jp

***Current address:** Department of Drug Disposition and Pharmacokinetics, School of Pharmaceutical Sciences, Teikyo University, Kanagawa, Japan

Materials and Methods

Materials

Morphine hydrochloride was purchased from Takeda Chemical Industries Ltd (Osaka, Japan). All other chemicals were purchased from commercial sources.

Animals

Male Sprague-Dawley rats weighing about 250 g were housed three or four per cage with free access to food and water and were maintained in a 12 h light–dark cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%).

This study was conducted in accordance with the guide for care and use of laboratory animals adopted by the US National Institutes of Health and guidelines approved by the Experimental Animal Ethical Committee of the University of Shizuoka.

Antinociceptive test

The tail-immersion test was used to quantify antinociception.[11] Briefly, the tail was immersed in a water bath at 55 ± 1°C and the time taken to withdraw the tail measured, termed the tail-flick latency. Baseline antinociceptive testing was performed before drug administration.

Rats were given oral morphine (30 mg/kg) 30 min after the oral administration of vehicle or quinidine (30 mg/kg). Antinociceptive testing was performed every 30 min for 180 min after morphine administration. A maximum tail-flick latency of 10 s was allowed in order to minimise tissue damage to the tail.

Values for tail-flick latency were converted to a percentage of the maximum possible effect (MPE): % MPE = (postdrug latency – predrug latency)/(maximum latency – predrug latency) × 100.

Measurement of morphine in plasma and brain

Rats were given morphine orally (30 mg/kg) or intravenously (30 mg/kg over 10 min via the femoral vein) 30 min after the oral administration of quinidine (30 mg/kg) or vehicle. Small amounts of blood (200 μl) were taken from the femoral artery 5, 10, 20, 30, 45, 60, 75, 90, 120 and 180 min later. The plasma was separated by centrifugation.

The brain was removed 60 or 180 min after morphine administration, weighed and then homogenised in four volumes of saline. The homogenate was centrifuged at 4800*g* for 20 min at 4°C. The supernatant and plasma sample were stored at −20°C until the measurement of morphine concentrations.

Concentrations of morphine were measured by HPLC with electrochemical detection.[12] Plasma and brain homogenate were subjected to solid-phase extraction using an Oasis HLB cartridge (Waters, Milford, MA, USA). The cartridge was pre-wetted with methanol (1 ml), followed by 10% acetonitrile in 20 mM phosphate buffer (pH 2.1) (1 ml) and water (1 ml). After the addition of 0.5 M ammonium sulfate buffer (pH 9.3) (900 μl), the plasma sample (50–100 μl) or supernatant of brain homogenate (100 μl) was applied to the cartridge, which was subsequently washed with 5 ml 5 mM ammonium sulfate buffer and 4 ml 15% methanol in 5 mM ammonium sulfate buffer and 0.5 ml water. The morphine was then eluted with 10% acetonitrile in 20 mM phosphate buffer (pH 2.1).

The HPLC system consisted of a pump (880-PU, Japan Spectroscopic Co. Jasco, Tokyo, Japan), an electrochemical detector (Nanospace SI-02, Shiseido, Tokyo, Japan) and an integrator (Chromatocorder 12, System Instruments, Tokyo, Japan). The column was a Capcell pak C_{18} ODS (4.6 mm × 250 mm, 5 μm particle size, Shiseido). The mobile phase was 25% acetonitrile, 2.5 mM sodium dodecyl sulfate and 50 mM phosphate buffer (pH 2.1), delivered at a flow rate of 0.7 ml/min. The limits of quantification of morphine in plasma and brain were 0.5 μM and 0.1 nmol/g brain tissue, respectively. Runs were accepted if the precision and accuracy of the quality control samples had a coefficient of variation below 15%.

Data analysis

Total body clearance (CL_{total}) of morphine was calculated from the area under the plasma concentration–time curve (AUC) after intravenous administration (CL_{total} = dose/AUC).

Data were compared using Student's *t*-test and one-way analysis of variance followed by Dunnett's test for single and multiple comparisons, respectively. A P value below 0.05 was considered significant.

Results

Antinociceptive effects

The antinociceptive effect of oral morphine determined by the tail-immersion test in vehicle- or quinidine-pretreated rats is shown in Figure 1. The antinociceptive effect of morphine was 19–27% of the MPE in vehicle-treated rats. Prior administration of quinidine markedly increased the antinociceptive effect of morphine, to 46–84% MPE. The area under the antinociceptive effect–time curve ($AUEC_{0–180}$) was

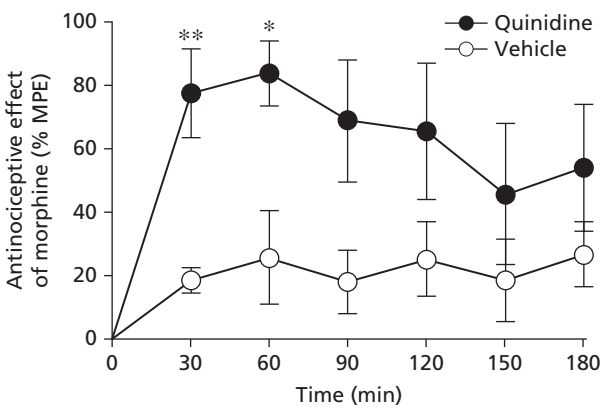

Figure 1 Effects of oral quinidine on the antinociceptive effect of morphine in rats, measured by tail-flick latency. Rats were treated with morphine (30 mg/kg p.o.) 30 min after the oral administration of vehicle or quinidine (30 mg/kg). Points are mean ± SE (n = 4 or 5 rats). *P < 0.05; **P < 0.01 vs vehicle-treated rats. MPE, maximum possible effect.

3.1-fold greater in quinidine-pretreated rats than in vehicle-treated rats: 11068 ± 2662 vs 3597 ± 1663% MPE · min (mean ± SE, n = 4 and 5, respectively).

Plasma and brain concentrations of morphine after oral administration

Plasma concentrations of morphine after oral administration to rats pretreated with vehicle or quinidine are shown in Figure 2. The plasma concentration of morphine in vehicle-pretreated rats reached a maximum (1.1 μM) 10 min after administration, and was 0.52–0.79 μM at 20–180 min. In quinidine-pretreated rats, the plasma concentration was 5.9 μM at 20 min, gradually decreasing to 2.2–2.3 μM at 120–180 min. The plasma concentration was markedly higher in the quinidine-pretreated rats than in the vehicle-treated controls at all time points. The AUC after oral dosing ($AUC_{0–180\ p.o.}$) was also larger (5.2-fold) in the quinidine-pretreated animals (Table 1).

The concentration of morphine in the brain in quinidine-pretreated rats was increased 2.8 fold at 60 min and 2.7 fold at 180 min compared with vehicle-treated rats (Figure 3).

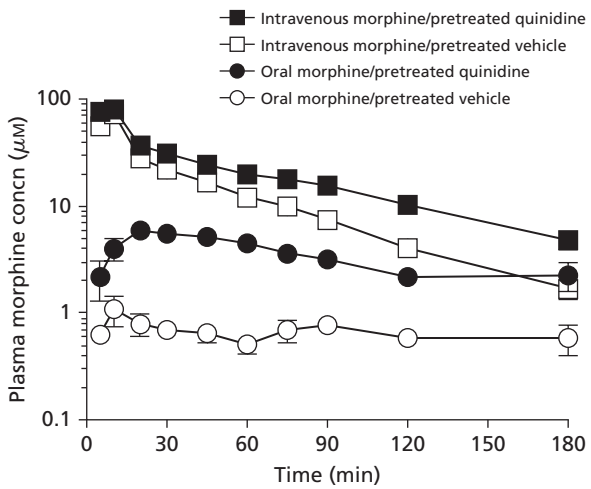

Figure 2 Plasma morphine concentrations following oral or intravenous administration to rats. Morphine was administered orally (30 mg/kg; circles) or intravenously (30 mg/kg over 10 min; squares) to rats pretreated with vehicle or quinidine (30 mg/kg p.o.). Points are means ± SD (n = 3 or 4 rats).

Table 1 Pharmacokinetic parameters calculated from plasma concentrations of morphine after oral (30 mg/kg) or intravenous (30 mg/kg over 10 min) administration in rats pretreated with vehicle and quinidine (30 mg/kg, p.o.)

	Vehicle	Quinidine
$AUC_{0–180\ p.o.}$ (μM · min)	117 ± 8	605 ± 57 (5.2)***
$AUC_{0–180\ i.v.}$ (μM · min)	2364 ± 37	3646 ± 235 (1.5)**
$AUC_{0–\infty\ i.v.}$ (μM · min)	2466 ± 48	4049 ± 346 (1.7)*
Volume of distribution (l/kg)	2.51 ± 0.20	2.01 ± 0.18
Total clearance (l/min per kg)	0.043 ± 0.001	0.026 ± 0.002 (0.60)**

AUC, area under the plasma concentration–time curve. Values represents means ± SE (n = 3 or 4 rats). Values in parentheses are ratios of the value in quinidine-pretreated rats to that in vehicle-pretreated rats. *P < 0.05; **P < 0.01; ***P < 0.001 vs vehicle-pretreated rats.

The brain: plasma concentration ratio at these time points was altered little by quinidine treatment (Figure 3).

Pharmacokinetics of morphine

The concentration of morphine in plasma was determined after intravenous infusion (30 mg/kg over 10 min). The plasma concentration of morphine was significantly higher in quinidine-pretreated rats than in vehicle-pretreated rats and values for the AUC ($AUC_{0–\infty\ i.v}$) were increased 1.7-fold (Figure 1 and Table 1). Quinidine treatment significantly decreased CL_{total} of morphine (by 40%) but did not change the volume of distribution. The AUC ratio ($AUC_{0–180\ p.o.}$/$AUC_{0–180\ i.v.}$) was significantly greater (3.3 fold) in quinidine-pretreated rats than in vehicle-pretreated rats: 0.166 ± 0.016 vs 0.0496 ± 0.0033 (mean ± SE, n = 3 and 4, respectively).

Discussion

Oral administration of quinidine significantly increased the absorption of morphine from the gastrointestinal tract, and subsequently enhanced the concentration in the brain and antinociceptive effect of morphine.

The antinociceptive effect of morphine (30 mg/kg p.o.) was increased by prior administration of quinidine (30 mg/kg p.o.), with a 3.1-fold greater $AUEC_{0–180}$ than that in vehicle-treated rats (Figure 1). The prior administration of quinidine increased the plasma concentration of morphine, with a 5.2-fold greater $AUC_{0–180\ p.o.}$ than that in vehicle-treated rats (Figure 2 and Table 1). Furthermore, the concentration of morphine in the brain was significantly increased by prior administration of quinidine (Figure 3). These results indicate that quinidine treatment increases the concentration of morphine in plasma and the brain, potentiating its antinociceptive effect.

Quinidine has a local anaesthetic effect like other class I antiarrhythmic drugs, but its systemic administration has no analgesic effect.[13] Thus, enhancement of the antinociception of morphine is likely to be due mainly to pharmacokinetic interaction rather than a pharmacodynamic interaction. It is unlikely that morphine-6-glucuronide, the active metabolite of morphine, contributes to the morphine antinociception because this route of metabolism is negligible in the rat.[14]

We further analysed the effects of quinidine on the pharmacokinetics of morphine including its absorption, systemic clearance and distribution in the brain. The AUC ratio ($AUC_{0–180\ p.o.}$/$AUC_{0–180\ i.v}$) was 3.3-fold greater in quinidine-pretreated rats than in vehicle-pretreated rats. The increase in AUC ratio caused by quinidine is consistent with the result of a clinical study in which quinidine caused a 1.6-fold increase in the oral AUC of morphine.[10] Quinidine is an inhibitor of P-glycoprotein, with an IC50 of 2.2 μM in Caco-2 cells.[15] The concentration of quinidine in intestinal fluid is estimated to be 2 mM, assuming that the volume of rat gastrointestinal fluid is 5 ml. Thus, quinidine should remain in the intestinal fluid at a concentration high enough to inhibit P-glycoprotein-mediated efflux. Crowe[16] suggested that absorption of morphine in Caco-2 cells could be doubled by the inhibition of P-glycoprotein. Taken together, the inhibition of intestinal P-glycoprotein by quinidine might be

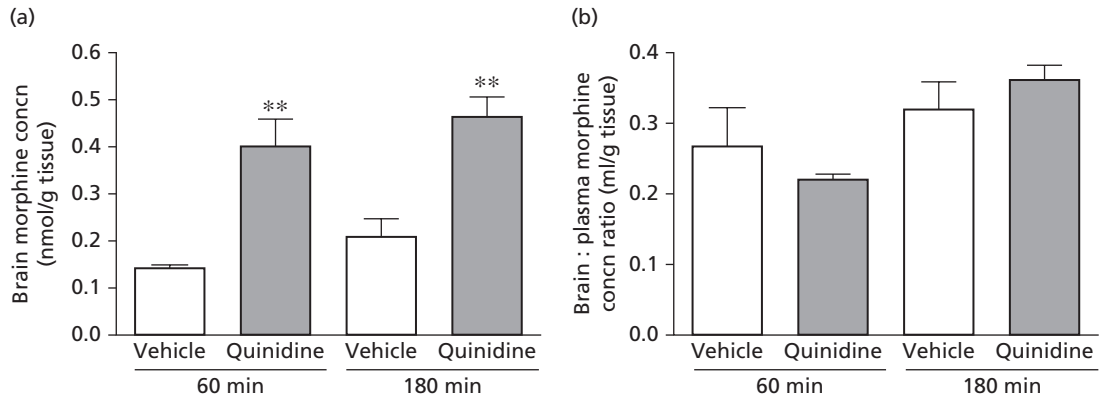

Figure 3 Brain morphine concentrations (a) and brain : plasma concentration ratios of morphine (b) following oral administration to rats pretreated with vehicle or quinidine. Columns represent means ± SE (n = 3–5 rats). $^{**}P < 0.01$ vs vehicle-treated rats.

one of the major reasons for the increase in the plasma concentration of morphine.

The administration of quinidine significantly increased the plasma concentration of morphine following intravenous infusion but without any increase in the volume of distribution, indicating a decrease in systemic clearance. This finding is in contrast to a clinical study showing that quinidine did not change the systemic clearance of morphine.[10] About 17% of the dose of morphine administered intravenously in rats is excreted unchanged in the urine,[17] and the primary route of metabolism is via glucuronidation. Quinidine inhibits several drug metabolism enzymes in addition to P-glycoprotein. Uchaipichat and colleagues[18] have reported that quinidine inhibits human UDP-glucuronosyltransferase 2B7, which metabolises morphine to its glucuronides, with a Ki value of 186 μM. However, it is unclear whether quinidine inhibits the glucuronidation of morphine *in vivo* in rats. Another possible reason for the decrease in CL_{total} is stimulation of enterohepatic recirculation. In rats, bile duct ligation decreased the plasma concentration of morphine after intravenous administration, enhancing the systemic clearance.[17] These results suggest that approximately 20% of the intravenous dose is subject to enterohepatic recirculation. Administration of quinidine may enhance the intestinal reabsorption of orally administered morphine, leading to an increase in the plasma concentration and a decrease in systemic clearance. Further studies to elucidate the mechanisms responsible for the decrease in the systemic clearance of morphine caused by quinidine administration in rats are required.

P-glycoprotein in the blood–brain barrier modulates the antinociceptive effect of morphine by regulating its transport from blood into the central nervous system.[5–8] The concentration of morphine in the brain was increased by the administration of quinidine but the brain : plasma concentration ratio was not changed. These results suggest that quinidine treatment does not affect the transfer of morphine from the bloodstream into the brain. However, the concentration of quinidine in the plasma in this study may not be high enough to inhibit P-glycoprotein in the blood–brain barrier. Indeed, based on the pharmacokinetic parameters for quinidine in rats,[19] we estimate that the plasma concentration of unbound quinidine 30 min after administration (30 mg/kg) was less than 1 μM, which is lower than the IC50 value for inhibition of P-glycoprotein-mediated transport by quinidine (2.2 μM).[15]

Conclusions

Quinidine increases the absorption of morphine from the gastrointestinal tract, and subsequently enhances its concentration in the brain and antinociceptive effect. Enhanced intestinal absorption of morphine may be partly attributed to inhibition of intestinal P-glycoprotein. P-glycoprotein inhibitors such as quinidine may enhance the pain-relieving effects of morphine.

Declarations

Conflict of Interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported in part by a Grant-in-Aid for Young Scientists provided by The Ministry of Education, Culture, Sports, Science and Technology.

Acknowledgement

The authors would like to thank Ms Ai Nakaiso for her technical assistance.

References

1. Klepstad P *et al*. Research Steering Committee of the EAPC. Pain and pain treatments in European palliative care units. A cross sectional survey from the European Association for Palliative Care Research Network. *Palliat Med* 2005; 19: 442–443.
2. Hoskin PJ *et al*. The bioavailability and pharmacokinetics of morphine after intravenous, oral and buccal administration in healthy volunteers. *Br J Clin Pharmacol* 1989; 27: 499–505.
3. Biancofiore G. Oxycodone controlled release in cancer pain management. *Ther Clin Risk Manag* 2006; 2: 229–234.

4. Peckham EM, Traynor JR. Comparison of the antinociceptive response to morphine and morphine-like compounds in male and female Sprague-Dawley rats. *J Pharmacol Exp Ther* 2006; 316: 1195–1201.
5. Schinkel AH *et al*. Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest* 1995; 96: 1698–1705.
6. Xie R *et al*. The role of P-glycoprotein in blood-brain barrier transport of morphine: transcortical microdialysis studies in mdr1a (–/–) and mdr1a (+/+) mice. *Br J Pharmacol* 1999; 128: 563–568.
7. Letrent SP *et al*. Effects of a potent and specific P-glycoprotein inhibitor on the blood-brain barrier distribution and antinociceptive effect of morphine in the rat. *Drug Metab Dispos* 1999; 270: 827–834.
8. Zong J, Pollack GM. Morphine antinociception is enhanced in mdr1a gene-deficient mice. *Pharm Res* 2000; 17: 749–753.
9. Boström E *et al*. Oxycodone pharmacokinetics and pharmacodynamics in the rat in the presence of the P-glycoprotein inhibitor PSC833. *J Pharm Sci* 2005; 94: 1060–1066.
10. Kharasch ED *et al*. Role of P-glycoprotein in the intestinal absorption and clinical effects of morphine. *Clin Pharmacol Ther* 2003; 74: 543–554.
11. Abe K *et al*. Characterization of muscarinic receptor subtypes in the rostral ventrolateral medulla and effects on morphine-induced antinociception in rats. *Eur J Pharmacol* 2003; 465: 237–249.
12. Okura T *et al*. Comparative measurement of spinal CSF microdialysate concentrations and concomitant antinociception of morphine and morphine-6β-glucuronide in rats. *Life Sci* 2007; 80: 1319–1326.
13. Tzeng JI *et al*. The cutaneous analgesic effect of class I antiarrhythmic drugs. *Anesth Analg* 2007; 104: 955–958.
14. Wu D *et al*. Blood-brain barrier permeability to morphine-6-glucuronide is markedly reduced compared with morphine. *Drug Metab Dispos* 1997; 25: 768–771.
15. Ekins S *et al*. Three-dimensional quantitative structure-activity relationships of inhibitors of P-glycoprotein. *Mol Pharmacol* 2002; 61: 964–973.
16. Crowe A. The influence of P-glycoprotein on morphine transport in Caco-2 cells. Comparison with paclitaxel. *Eur J Pharmacol* 2002; 440: 7–16.
17. Horton TL, Pollack GM. Enterohepatic recirculation and renal metabolism of morphine in the rat. *J Pharm Sci* 1991; 80: 1147–1152.
18. Uchaipichat V *et al*. Selectivity of substrate (trifluoperazine) and inhibitor (amitriptyline, androsterone, canrenoic acid, hecogenin, phenylbutazone, quinidine, quinine, and sulfinpyrazone) "probes" for human udp-glucuronosyltransferases. *Drug Metab Dispos* 2006; 34: 449–456.
19. Sugihara *et al*. The influence of increased plasma protein binding on the disposition of quinidine in turpentine-treated rats. *Biol Pharm Bull* 1993; 16: 63–67.