Enhancement by Grapefruit Juice of Morphine Antinociception

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Drug–food interactions are increasingly recognized as noteworthy clinical events that should be considered in order to avoid adverse effects. Indeed, the intake of grapefruit juice has been demonstrated to elevate serum concentrations of several drugs including calcium channel blockers such as felodipine, nifedipine and nisoldipine, verapamil, cyclosporine, tacrolimus and midazolam.1–6) The main mechanism for the interaction with grapefruit juice is considered to be the inhibition of cytochrome P450 3A4 (CYP3A4), the major drug metabolism enzyme in the intestine. Recent investigations have shown that grapefruit juice inhibits not only CYP3A4 but also drug transporters like P-glycoprotein,7–9) which plays important roles in the intestinal barrier function in a coordinated manner with CYP3A4.10) The inhibitory effect of grapefruit juice on the intestinal barrier function may enhance the oral bioavailability of drugs, which has been associated with a higher incidence of side effects. From the point of view of beneficial use of dietary constituents, the enhancement of bioavailability can potentiate the therapeutic effect of drugs. Until now, few studies have focused on the beneficial use of interaction between dietary constituents and drugs.

Morphine is the most commonly used opioid analgesic for the treatment of cancer pain. Morphine is a substrate of P-glycoprotein,11) and its antinociceptive effect is enhanced by the inhibition of cytochrome P450 3A4 (CYP3A4),12–14) and its antinociceptive effect is enhanced by the oral administration of grapefruit juice (2 ml/rat). Further, the effect of grapefruit juice was examined in morphine-tolerant rats. The repeated administration of morphine (100 mg/kg p.o.) for 5 d caused a marked decrease in the antinociceptive effect of morphine. To examine the pharmacokinetics of morphine after the repeated treatment with morphine for 5 d, microdialysis probes were implanted into the jugular vein and spinal intrathecal space in rats. The morphine concentrations in the blood and intrathecal cerebrospinal fluid (CSF) were gradually decreased by the repeated treatment with morphine. The grapefruit juice treatment significantly increased the blood concentration of morphine in morphine-tolerant rats. These results suggest that oral administration of grapefruit juice enhances the morphine antinociception by increasing the intestinal absorption of this agent.

Key words morphine; grapefruit juice; tolerance; microdialysis

The aim of this study was to investigate the effect of grapefruit juice intake on the antinociception of morphine in rats. The antinociception of morphine (30 mg/kg, per os (p.o.)) was significantly enhanced by the oral administration of grapefruit juice (2 ml/rat). Further, the effect of grapefruit juice was examined in morphine-tolerant rats. The repeated administration of morphine (100 mg/kg p.o.) for 5 d caused a marked decrease in the antinociceptive effect of morphine, indicating the development of morphine-tolerance. In the morphine-tolerant rats, oral administration of grapefruit juice potentiated significantly the antinociceptive effect of morphine. The grapefruit juice treatment significantly increased the blood concentration of morphine in morphine-tolerant rats. These results suggest that oral administration of grapefruit juice enhances the morphine antinociception by increasing the intestinal absorption of this agent.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing about 250 g were housed three to four per cage with free access to food and water and maintained on a 12-h light/dark cycle in a room with controlled temperature (24±1 °C) and humidity (55± 5%) throughout a whole experimental period. This study was conducted according to guidelines approved by the Experimental Animal Ethical Committee of University of Shizuoka. Morphine hydrochloride was purchased from Takeda Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were purchased from commercial sources.

Grapefruit juice (Sunkist®) was orally administered at a volume of 2 ml per rat 30 min prior to the drug administration in accordance with previous report.15) In the single administration experiment, rats received 2 ml of water, grapefruit juice (Sunkist®) or quinidine (30 mg/kg per os (p.o.)), a P-glycoprotein inhibitor,18) and 30 min later, morphine (30 mg/kg). The tail-flick latency test was used to quantify antinociception, with a thermal stimulus being applied to the tail.19) Before the drug administration, baseline antinociceptive testing was performed. The antinociceptive testing was performed at 60, 120 and 180 min after the morphine treatment. A maximum tail-flick latency of 10 s was used to minimize the tissue damage to the tail. The tail-flick latency values were converted to a percentage of the maximum possible effect (%MPE): %MPE=(postdrug latency−predrug latency)/(maximum latency−predrug latency)×100.

In the repeated administration experiment, development of morphine tolerance was measured according to a method described previously with a slight modification. Briefly, rats received morphine (100 mg/kg) orally once a day for 5 d, and...
antinociception was determined by tail-flick test as described above once a day (120 min after morphine administration) to avoid tissue damage by repeated measurements. On the day (day 6) after the last treatment, 2 ml of grapefruit juice (Sunkist®) was orally administered to rats 30 min before another administration of morphine (100 mg/kg p.o.), and the tail-flick latency test was performed as described above.

The concentration of morphine in the blood and spinal CSF was determined by the microdialysis method. The spinal intrathecal dialysis probe was constructed from Cuprophan hollow fibers (inside diameter (i.d.), 0.2 mm; MW cut-off, 12500; RENAK-E, RE-10M, Kawasumi Chemical Industries Ltd., Tokyo, Japan). The fibers were coated with epoxy glue, except for a 4-cm region in the middle. A Nichrome wire (outside diameter (o.d.), 0.1 mm; Unique Medical Co., Ltd., Tokyo, Japan) was then passed through the fiber and both ends of the fiber were attached to pieces of polyethylene tube (PE-10; Natsume Seisakusho Co., Ltd., Tokyo, Japan). Rats were anesthetized with ketamine (188 mg/kg intramuscular injection (i.m.)) and their heads were placed in a stereotaxic apparatus (SR-6, Narishige Scientific Instrument Lab., Tokyo, Japan). The probe was inserted through an incision in the cisternal membrane and slowly passed caudally 9 cm into the intrathecal space to leave the uncoated section of the catheter at the Th11–L2 spinal segments. The two PE-10 ends of the dialysis probe were externalized on the top of the head. Rats were allowed to recover from the surgery for 3 d. They were then anesthetized with ether and a dialysis probe for vessels (TP-100-10, Eicom Corp., Tokyo, Japan) was implanted into the jugular vein. The dialysis probes implanted into the jugular vein and spinal intrathecal space were perfused at a constant rate of 5 μl/min with Ringer’s solution (147 mM NaCl, 4 mM KCl, 2.4 mM CaCl₂, pH 7.3) containing antipyrine as a reference of probe recovery. After the oral administration of morphine (100 mg/kg) in rats, collection of the dialysate was started. The blood and spinal CSF dialysate samples were collected every 60 min for 300 min, and each sample was kept at ~20°C until the analysis. The dialysate concentration of morphine was measured by HPLC with fluorimetric detection. The HPLC system consisted of a pump (880-PU, Japan Spectroscopic Co., (Jasco), Tokyo, Japan), a fluorescence detector (RF-535, Shimadzu, Tokyo, Japan) and an integrator (C-R6A, Shimadzu, Tokyo, Japan). The analytical column was composed of Nucleosil C18 ODS (4.6 mm×250 mm, 5 μm particle size, GL Sciences). Gradient elution was carried out at room temperature at a constant flow rate of 1.0 ml/min. Solvent A was 0.1% TFA in water and solvent B was 0.1% TFA in 40% acetonitrile. The initial concentration of acetonitrile was 6.4%. After the injection of sample, the system was pumped isocratically for 2 min, followed by a gradient from 6.4 to 20% acetonitrile over 10 min, and then a gradient from 20 to 40% acetonitrile over 2 min to wash the column. The column elute was monitored fluorometrically at excitation and emission wavelengths of 280 and 335 nm, respectively. The concentration of morphine in blood (C\textsubscript{blood}) or spinal CSF (C\textsubscript{CSF}) was estimated from the dialysate concentration (C\textsubscript{d}) using antipyrine as a reference.

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C_{\text{blood}} \text{ or } C_{\text{CSF}} = C_d \cdot \left(1 - \exp(-R_{\text{dref}} PA_{\text{vivo}}/F)\right)
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F is the dialysate flow rate and PA\textsubscript{vivo} is the \textit{in vitro} permeability rate constant, which can be estimated from the \textit{in vitro} recovery of the microdialysis probe. \(R_{\text{dref}}\) is the effective dialysis coefficient of the reference compound, antipyrine, which is the ratio of the \textit{in vivo} and \textit{in vitro} probe recovery.

The statistical analysis of the data was performed with Student’s t-test for single comparisons. Differences were considered statistically significant at \(p<0.05\).

RESULTS AND DISCUSSION

In the single administration experiment, the morphine caused increases in latency in the tail-flick test in rats. The oral administration of grapefruit juice (2 ml/rat p.o. 30 min before the morphine administration) significantly increased the antinociception at 60 min after the morphine was administered (Fig. 1). The area under the effect–time curve (AUE) in grapefruit juice-treated rats was 1.5-times greater than that in control rats. The oral administration of quinidine, a P-glycoprotein inhibitor, increased markedly antinociception of morphine as shown by 2.8-fold greater AUE compared that in control rats (Fig. 2). On the other hand, the administration of grapefruit juice without morphine did not cause antinociceptive effects (data not shown). These results suggest that grapefruit juice enhances the antinociceptive effect of morphine in rats, though the antinociception increase by grapefruit juice was smaller than that by quinidine.

In the repeated administration experiment, the rats received morphine (100 mg/kg) orally once a day for 5 d. An antinociception was measured by the tail-flick test 120 min after receiving morphine. The antinociception was 100%
Morphine (100 mg/kg) was given orally once a day for 1—5 d. After 120 min, rats were subjected to the tail-flick test. On the day (day 6) after the 5-d-treatment with morphine, rats received grapefruit juice (GFJ) (2 ml/rat) 30 min before receiving morphine. The data are presented as % MPE. Each column represents the mean±S.E. for four rats. *p<0.05 vs. day 5.

Microdialysis probes were implanted into the jugular vein and intrathecal space. Rats received orally morphine (100 mg/kg). After the morphine administration, dialysate samples were collected every 60 min for 300 min, and the concentrations were measured. Each column represents the mean±S.E. for four rats.

MPE on day 1 and 2, and 75, 25 and 14% MPE, respectively, on day 3, 4 and 5 (Fig. 3). Tolerance developed with the repeated oral administration of morphine. The day (day 6) after the 5-d-treatment with morphine, rats were administered grapefruit juice (2 ml/rat p.o.) 30 min before receiving morphine. The pretreatment with grapefruit juice significantly enhanced the antinociception of morphine from 14% MPE (day 5) to 48% MPE.

To determine the pharmacokinetics of morphine during the development of morphine-tolerance, a microdialysis method was applied to the jugular vein and spinal intrathecal space. The concentrations of morphine in blood and spinal CSF on day 1 increased with time and reached maximum levels at 180—240 min in blood and 120—180 min in spinal CSF, respectively (Fig. 4). The concentrations of morphine in blood and spinal CSF gradually decreased during the oral treatment (Fig. 5). The day (day 6) after the 5-d-treatment with morphine, rats were administered grapefruit juice (2 ml/rat p.o.) 30 min before receiving morphine. The $AUC_{\text{blood}}$ was significantly (1.9 times) greater on day 6 than day 5 (Fig. 5A). The $AUC_{\text{CSF}}$ was increased 1.3 times by the grapefruit juice, but not significantly (Fig. 5B). The concentration ratio of $AUC_{\text{CSF}}$ to $AUC_{\text{blood}}$ ($AUC_{\text{CSF}}/AUC_{\text{blood}}$) was 0.62, 0.45, 0.67 and 0.50 on day 1, 3, 5 and 6, respectively. The increases in the plasma concentration of morphine caused by grapefruit juice may contribute at least partly to the enhancement of morphine antinociception.

Grapefruit juice and its constituents are considered to affect the functions of drug transporters such as P-glycoprotein,7—9 multir drug resistance protein 2 (MRP2)25 and organic anion transporting polypeptide (OATP),26 in addition to the drug metabolism enzyme CYP3A4. Of these enzymes and transporters, P-glycoprotein can affect morphine’s disposition, because morphine is a substrate for P-glycoprotein, but not CYP3A4 or MRP2.16—19 It was reported that the inhibition of intestinal P-glycoprotein by oral administration of quinidine enhances the absorption and pharmacological effect of morphine in humans.15 Oral administration of quinidine also elevated the antinociception of orally-administered morphine in rats. Grapefruit juice extracts cause a four-fold increase in the transport of [3H]vinblastine, a P-glycoprotein substrate, from apical to basolateral sides across human intestinal Caco-2 cells.8 De Castro et al.9 have reported that 6,7-epoxybergamottin, 6,7-dihydroxybergamottin, naringin and naringenin in grapefruit juice inhibit the P-glycoprotein-mediated transport of talinolol in human intestinal Caco-2 cells with IC50 values of 0.7, 34, 236 and 2409 μM, respectively. They have suggested that these furanocoumarins and flavonoids are able to inhibit intestinal P-glycoprotein-mediated transport because they are present in grapefruit juice in the same concentration ranges.27,28 Taken together, oral administration of grapefruit juice may potentiate the antinociceptive effect of morphine by increasing the intestinal absorption possibly via the inhibition of intestinal P-glycoprotein.

P-glycoprotein at the blood—brain barrier modulates the antinociceptive effect of morphine by regulating its transport from the blood into the central nervous system.12—14 However, the spinal CSF to blood concentration ratio of morphine was not changed by grapefruit juice, suggesting little or insignificant inhibition of P-glycoprotein at the brain barrier. Although flavonoids and furanocoumarins are partially absorbed from the intestine, flavonoids such as naringin are most likely hydrolyzed by intestinal enzymes29,30 and bergamottin has very low permeability through CYP3A4-expressing Caco-2 cell monolayers.31 In addition, bergamottin and dihydroxybergamottin strongly bind to human serum albumin. These dispositional properties may explain in part why grapefruit juice inhibits intestinal CYP3A4 rather than hepatic CYP3A4 in vivo.32 The unbound concentrations of these flavonoids and furanocoumarins in blood may be too low to inhibit P-glycoprotein at the luminal membrane of the blood—brain barrier.

Aquilante et al.16 have reported that repeated morphine...
administration causes a two-fold increase in the P-glycoprotein level in rat brain associated with the decrease in the antinociceptive effect. In morphine-tolerant rats, intestinal P-glycoprotein-mediated transport may be stimulated and therefore more susceptible to the inhibition of intestinal P-glycoprotein. Thus, inhibitors of intestinal P-glycoprotein such as grapefruit juice may partly overcome morphine-tolerance, though little clinical evidence has been presently reported on enhancement of effects of morphine by grapefruit juice. In fact, it may be difficult to control the intestinal P-glycoprotein activity using grapefruit juice, because the amounts of flavonoids and furanocoumarins may differ with area, season, and production process.\(^{27,28}\) In addition, it has been suggested that grapefruit juice–drug interaction is caused by additive or synergistic effects of several flavonoids and furanocoumarins in the juice.\(^ {32}\) Thus, further quantitative analysis will be required to clarify the mechanism underlying enhancement of the antinociception of morphine by grapefruit juice.

In conclusion, grapefruit juice is suggested to potentiate the antinociception of morphine associated with an increase in intestinal absorption. This enhancement may partially overcome morphine-tolerance.

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REFERENCES AND NOTES

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