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Quinine-induced inhibition of gastrointestinal transit in mice: possible involvement of endogenous opioids

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Abstract

The effect of quinine, a cinchona alkaloid, was studied on gastrointestinal transit in mice. Intraperitoneal (i.p.) administration of quinine inhibited the intestinal propulsion of a charcoal suspension at a dose of 100 mg/kg, comparing favorably with 5 mg/kg morphine. In an attempt to probe into the mechanism underlying this inhibition, a possible modulation by minoxidil (1 mg/kg, p.o.) and glibenclamide (1 mg/kg, p.o.), the drugs that, respectively, open and close ATP-sensitive K^+ channels was tested on gastrointestinal transit in animals treated or not with quinine or morphine. While minoxidil produced no significant change of normal transit, glibenclamide significantly increased it. However, both drugs blocked the quinine-induced reduction in gastrointestinal transit. In contrast, the inhibitory effect of morphine on gastrointestinal transit was not modified by either drug. The effects of quinine as well as of morphine on gastrointestinal transit were significantly antagonized by naloxone (2 mg/kg, s.c.), a μ-opioid receptor antagonist but not by yohimbine (1 mg/kg, i.p.), an α_2-adrenoceptor antagonist. Furthermore, quinine at a lower dose (25 mg/kg) that showed no per se effect on gastrointestinal transit, significantly potentiated the response to 2.5 mg/kg morphine. Although the role of ATP-sensitive K^+ channels in the action of quinine and morphine was not clarified by the present results, a possible involvement of endogenous opioid(s) in the quinine-induced inhibition of gastrointestinal transit can be suggested.

Keywords: Quinine; Intestinal transit; ATP-sensitive K^+ channel; Morphine; Yohimbine; Naloxone; (Mouse)

1. Introduction

Quinine, an alkaloid of cinchona bark, in spite of its cardiovascular toxicity, is still considered the drug of choice in severe chloroquine-resistant *Plasmodium falciparum* malaria (Wirima et al., 1990). It promotes parasite and fever clearance in cerebral malaria, and some of the mechanisms suggested include its interference with parasite metabolism and suppression of tumour necrosis factor-alpha (TNF-α) and prostaglandin generation (Zidovetzki et al., 1993; Gabay et al., 1994; Gantner et al., 1995; Santos and Rao, 1998). Quinine also a very frequently consumed beverage in the form of tonic water, has a long history of use for nearly three centuries. Despite the widespread consumption of this quininated beverage, there has been little research on its gastrointestinal effects and toxicity. In contrast, the cardiovascular toxicity of quinine, its antipyretic action and efficacy in cerebral malaria have been studied extensively in both experimental animals and the clinic (Wirima et al., 1990; Jacaz-Aigrain et al., 1994). Gastrointestinal symptoms such as nausea, vomittings and diarrhoea are prominent after large single oral doses of quinine and are also seen in cinchonism (Bateman and Dyson, 1986; White, 1992). Symptoms of thrombocytopenic purpurea may occur in some individuals following ingestion of tonic water (Tracy and Webster, 1996). A recent study in rats has shown that after they have tasted quinine, ad libitum feeding tends to increase amylase activity, the gastric pH is decreased and intestinal nitrogen contents stagnate (Ohara et al., 1996). To find out more about gastrointestinal effects, the present study compared the effect of quinine on gastrointestinal transit with that of morphine, an opioid alkaloid known for its depressant effect on intestinal transit and motility (Burks et al., 1988). Since quinine blocks a wide variety of K^+ channels (Rudy, 1988) and since morphine opens ATP-dependent K^+ channels (McFadzean, 1988), a possible modulatory influence

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Table 1
Effect of quinine and morphine on gastrointestinal transit in mice

Treatment (mg/kg)	Gastrointestinal transit (%)	Inhibition (%)
Saline	73.21 ± 1.55	
Quinine 25	70.97 ± 1.81	3
Quinine 50	66.95 ± 2.71	9
Quinine 100	31.97 ± 4.44[b]	56
Morphine 2.5	51.66 ± 6.14[a]	29
Morphine 5	29.22 ± 2.32[b]	60
Morphine 10	28.07 ± 3.47[b]	62
Quinine 25 + morphine 2.5	34.50 ± 5.39[b]	53

Quinine (i.p.) and morphine (s.c.) were administered 30 min before the charcoal suspension. Each value represents the mean ± S.E.M.
[a] $P < 0.05$; [b] $P < 0.01$ vs. saline group (Student–Newman–Keuls test).

of minoxidil and glibenclamide, the drugs that respectively, open and close ATP-sensitive K^+ channels (Boyd, 1988; Meisheri et al., 1988) was also tested on the intestinal action of quinine and morphine.

2. Materials and methods

2.1. Animals

Male Swiss albino mice, weighing 21–23 g were used. They were housed eight per cage under standard animal room conditions (temperature 21 ± 1°C; humidity 55–60%) on a 12-h light–12-h dark cycle with food and water continuously available for at least one week before the experiment. The animals were starved for 24 h before the experiment with free access to water. All the experimental procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Ceara, Fortaleza.

2.2. Gastrointestinal transit

The effect of quinine (25, 50 and 100 mg/kg, i.p.), morphine (2.5, 5 and 10 mg/kg, s.c.) or vehicle (normal saline, 5 ml/kg, i.p.) on gastrointestinal transit was studied in conscious mice as described by Janssen and Jageneau (1957). An aqueous charcoal suspension (2 ml/kg of a 10% activated charcoal suspension in 5% gum arabic) was given orally to each mouse, 30 min after saline or drug administration. The animals were killed by cervical dislocation under ethyl ether anesthesia 20 min after receiving the charcoal and the intestines were carefully removed without stretching and extended on a clean glass surface. The total length of the intestine from pylorus to ileo-caecal junction as well as the length travelled by the charcoal was measured. Gastrointestinal transit was expressed as the length travelled by the charcoal as a percentage of the total length of the small intestine. In some experiments, minoxidil (1 mg/kg, p.o.), glibenclamide (1 mg/kg, p.o.), naloxone (2 mg/kg, s.c.) or yohimbine (1 mg/kg, i.p.) was administered 15 min before quinine or morphine to study their influence on gastrointestinal transit. Doses of drugs and their administration-test intervals were selected based on existing literature and from our pilot experiments.

2.3. Chemicals and drugs

Quinine hydrochloride, yohimbine hydrochloride and naloxone hydrochloride were obtained from Sigma Chemical (St. Lous, MO, USA). The other drugs used were morphine sulphate (Dimorph®, Cristalia), minoxidil (Minoxidine®, Sanval) and glibenclamide (Daonil®, Hoechst). Minoxidil and glibenclamide were suspended in normal saline using 0.2% carboxymethylcellulose. All other drugs were dissolved in normal saline.

2.4. Statistical analysis

All data are given as means ± S.E.M. and were analysed by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test for multiple comparisons. P values of 0.05 or less were considered significant.

3. Results

3.1. Effect of quinine and morphine on gastrointestinal transit

Both quinine and morphine inhibited the intestinal propulsion of charcoal in mice (Table 1). The inhibitory effect of quinine on gastrointestinal transit was found to be

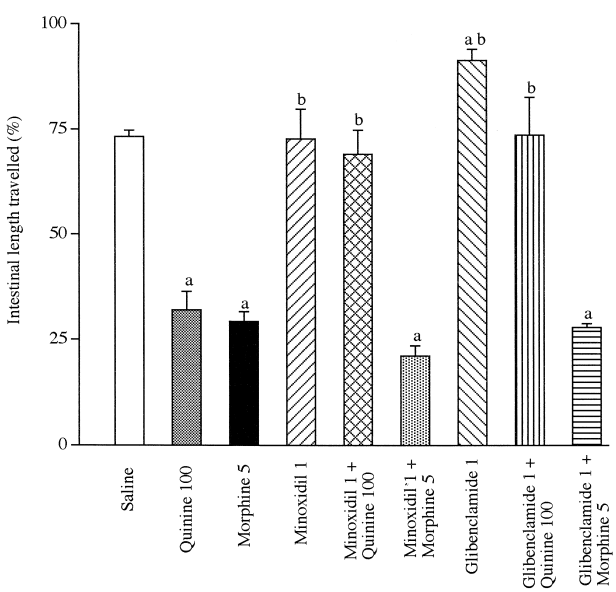

Fig. 1. Effects of ATP-sensitive K^+ channel drugs, minoxidil (1 mg/kg) and glibenclamide (1 mg/kg) on quinine (100 mg/kg) or morphine (5 mg/kg)-induced inhibition of small intestinal transit in mice as assessed by charcoal propulsion. Each column represents the means ± S.E.M. for eight animals. [a] $P < 0.01$ vs. saline controls. [b] $P < 0.01$ vs. quinine or morphine alone (ANOVA and Student–Newman–Keuls test).

significant at 100 mg/kg only (56% inhibition) but not at 25 and 50 mg/kg whereas morphine produced a dose-related significant inhibition. Inhibition was of the order of 29, 60 and 62%, for the respective doses of 2.5, 5 and 10 mg/kg. When a 25-mg/kg quinine dose that did not itself produce significant inhibition of gastrointestinal transit was coadministered with a low dose of morphine (2.5 mg/kg), the observed reduction (53%) was greater than the sum of that with the two drugs given individually (32%).

3.2. Effect of ATP-sensitive K^+ channel modulators on the inhibition of gastrointestinal transit caused by quinine and morphine

As shown in Fig. 1, the normal transit was not significantly influenced by minoxidil (1 mg/kg), the K^+ channel opener, but was increased by glibenclamide (1 mg/kg), the ATP-sensitive K^+ channel blocker. However, both minoxidil and glibenclamide were found to block the quinine-induced reduction of gastrointestinal transit. There was no significant change in the inhibitory effect of morphine on gastrointestinal transit in either the minoxidil- or glibenclamide-treated groups.

3.3. Effect of naloxone and yohimbine on the inhibition of gastrointestinal transit produced by quinine and morphine

The inhibitory effect of both quinine (100 mg/kg) and morphine (5 mg/kg) on gastrointestinal transit was significantly antagonized by naloxone (2 mg/kg) pretreatment (Fig. 2), but not by yohimbine (1 mg/kg), an α_2-adrenoceptor blocking agent (data not shown). Naloxone, at the dose tested, produced no significant effect on normal transit. However, naloxone was found to revert the anti-transit effect of quinine completely and that of morphine only partially.

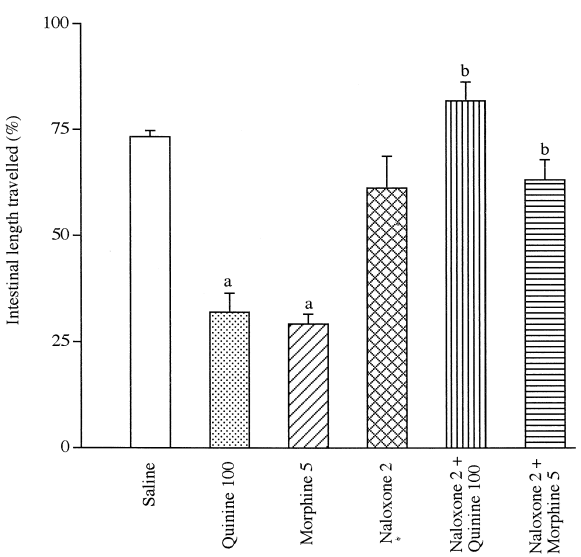

Fig. 2. Reversal by naloxone (2 mg/kg) of the inhibitory effects of quinine (100 mg/kg) and morphine (5 mg/kg) on small intestinal transit in mice as assessed by charcoal propulsion. Each column represents the means ± S.E.M. for eight animals. $^aP < 0.01$ vs. saline controls. $^bP < 0.01$ vs. quinine or morphine alone (ANOVA and Student–Newman–Keuls test).

4. Discussion

The present results show that quinine, like morphine delays intestinal transit in mice and this finding is consistent with data showing its ability to cause stasis of intestinal contents (Ohara et al., 1996). Evidence suggests that μ-opioid receptors contribute to the opioid-induced anti-transit effect in rodents (Burks et al., 1988), probably involving ATP-sensitive K^+ channels (Poggioli et al., 1995). Since quinine is known to block a broad range of voltage-gated membrane conductances in a variety of excitable tissues (Malchow et al., 1994), the present study results confirmed the μ-opioid receptor involvement and the likelihood of a role of ATP-sensitive K^+ channels in the anti-transit effect of quinine, as for morphine. The delay in transit caused by quinine and morphine was significantly blocked by naloxone, a μ-opioid receptor antagonist (Ramabadram and Bansinath, 1990) but not by yohimbine, an α_2-adrenoceptor antagonist (Starke et al., 1975), suggesting an opioid mechanism and not an adrenergic one.

The blockade by naloxone of the anti-transit effect of quinine cannot be explained only by a direct action of quinine on opioid receptors. A possible explanation could be that quinine stimulates the release of some endogenous μ-receptor-selective opioid peptide(s) like endorphins and endomorphins (Hughes et al., 1975; Zadina et al., 1997). The latter peptides, although not studied for their anti-transit effects, seem to mediate analgesia in mice with a potency similar to that of morphine (Zadina et al., 1997). In this study, a 2 mg/kg dose of naloxone was able to block the anti-transit effect of quinine more completely and that of morphine only partially. The sensitivity of naloxone to μ-opioid receptors is much higher at smaller doses whereas at higher doses it blocks more effectively δ- and κ-opioid receptors (Stein et al., 1989). The fact that the reversibility of the anti-transit effect of quinine by a low dose of naloxone suggests selective activation of μ-opioid receptor in its action.

High dose-administrations of quinine is generally associated with diarrhoea in humans (Bateman and Dyson, 1986; White, 1992). Quinine in excess dosage might contribute to clinical diarrhoea involving other mechanisms which remains to be established. Separation of antidiarrhoeic actions, and anti-transit effects, however, was evident from studies with a δ-opioid agonist, cyclic [D-Pen^2, D-Pen^2] enkephalin (DPDPE) which decreases diarrhoea but do not inhibit transit (Burks et al., 1988). The antidiarrhoeal effects of morphine and of DPDPE, have been

ascribed to centrally-directed inhibition of intestinal secretion of fluids and electrolytes rather than to anti-transit effects. Plausibly quinine action at least in mice may involve μ-opioid receptors that selectively inhibit intestinal transit.

The mechanism of the quinine-induced release of μ-receptor-selective peptides remains unclear. It could presumably result from two peripheral mechanisms of action, one being the stimulation of opioid release and the other a release of acetylcholine decreased by an effect within the neural circuitry of the enteric nervous system. Further, the observed synergistic effect between morphine and quinine seen at smaller doses may reflect an interaction at μ-opioid receptor sites or at the metabolic level. Exogenous morphine acting directly and quinine acting by stimulation of endogenous opioids, their synergistic anti-transit action on gastrointestinal tract may result from a μ-opioid receptor mediated opening of ATP-sensitive K^+ channels. Both drugs are alkaloidal in nature, but they are structurally different, i.e., quinine is a quinoline derivative whereas morphine, is a phenanthrene derivative. However, it is known that structurally distinct classes of drugs can exert pharmacological actions similar to those of morphine. We have noticed similar synergism in the analgesic effect of these agents in animal models of nociception (unpublished observations). The observed synergism between quinine and morphine may be useful in therapeutic situations to minimize the side effects and tolerance development associated with morphine use.

The hypotensive agent, minoxidil, and the antidiabetic drug, glibenclamide, are well known for their modulating effects on ATP-sensitive K^+ channels. Minoxidil activates ATP-modulated K^+ channels by opening K^+ channels in smooth muscle and thereby permitting potassium efflux, it causes hyperpolarization and relaxation of smooth muscle (Meisheri et al., 1988) whereas glibenclamide blocks ATP-sensitive K^+ channels thereby causing membrane depolarization and influx of calcium through voltage-sensitive Ca^{2+} channels (Boyd, 1988). ATP-sensitive K^+ channels are present in intestinal smooth muscle and epithelial cells, and are opened by drugs like cromokalim and pinacidil (Sun and Benishin, 1994; Poggioli et al., 1995) and blocked by glibenclamide (Franck et al., 1994). K^+ channel openers can hyperpolarize and relax intestinal smooth muscles, stimulate NaCl absorption in villus cells and they may exert antidiarrhoeal activity (Poggioli et al., 1995). Interestingly, the results of this study showed that minoxidil as well as glibenclamide, while they exerted no influence on morphine action, were able to reverse the inhibitory effect of quinine on gastrointestinal transit. This result was unexpected because minoxidil and glibenclamide are known to exert opposite effects on ATP-sensitive K^+ channels (Stein et al., 1997). Quinine is a potent blocker of Ca^2-activated and voltage-gated K^+ channels (Rudy, 1988). The delay in intestinal transit caused by quinine may be a consequence of its effect on Ca^2-activated K^+ channels. In support of this view were the findings of Lee et al. (1993) and Yamamoto et al. (1997) of a quinine-induced block of an acetylcholine-activated voltage current that involves Ca^2-activated K^+ channels.

Glibenclamide selectively blocks ATP-sensitive K^+ channels only but has no effect on either voltage-gated K^+ channels or Ca^2-activated K^+ channels (Light and French, 1994). Whether minoxidil has an effect on Ca^{2+}-activated K^+ channels is unknown. In contrast, both drugs are unable to reverse the morphine-induced decrease in gastrointestinal transit. There are conflicting reports on the involvement of ATP-dependent K^+ channels in the gastrointestinal effects of morphine (Patil and Thakker, 1996). Sunita et al. (1994) have reported that glibenclamide, which blocks ATP-sensitive K^+ channels, failed to antagonize the intestinal action of morphine, but pinacidil and cromakalim, the K^+ channel openers, can inhibit intestinal propulsion, comparing favorably with morphine in this respect (Poggioli et al., 1995). These discrepancies might result from the heterogenous nature of the major types of K^+ channels, the existence of several subtypes within each major type of K^+ channel (Bolton and Beech, 1992; Inagaki et al., 1996; Clement et al., 1997) and also possibly from variations in the expression of K^+ channels among tissues and organs. Further studies are required to clarify the exact role of ATP-sensitive K^+ channels in the anti-transit effect of morphine and quinine, using K^+ channel modulators other than minoxidil and glibenclamide.

In conclusion, this study has demonstrated that quinine delays small intestinal transit possibly involving μ-opioid receptors. In the gut opioids are biosynthesized and processed locally and μ-opioid receptors are found in the gastrointestinal wall (O'Donohue and Dorsa, 1982). Therefore we suggest that quinine stimulates μ-opioid receptors and delays intestinal transit through an opioid mechanism. This might also explain the long known ability of quinine to act as mild analgesic.

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