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Compounds possessing 5-HT_3 receptor antagonistic activity inhibit intestinal propulsion in mice

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Abstract

The role of 5-HT_3 receptors in the control of intestinal propulsive activity was investigated in mice by a simple method in which the time taken for excretion of the head of an orally administered non-absorbable marker (whole gut transit time) was measured. Selective 5-HT_3 receptor antagonists ramosetron (YM060) at 0.01–0.3 mg/kg s.c. and ondansetron at 0.1–1 mg/kg s.c. dose-dependently prolonged the whole gut transit time. Prokinetic benzamides, such as renzapride (0.3–10 mg/kg s.c.), zacopride (0.01–0.3 mg/kg s.c.) and cisapride (0.1–3 mg/kg s.c.), which have been reported to possess 5-HT_3 receptor blocking properties, also dose-dependently prolonged it. These results indicate that activation of 5-HT_3 receptors seems to be one factor that underlies the physiological control of intestinal propulsive activity in mice. In contrast to their beneficial therapeutic effects on gastroduodenal dysmotility, prokinetic benzamides, at least those which have 5-HT_3 receptor antagonistic activity, may be unsuitable in the treatment of impaired lower intestinal propulsive activity.

Keywords: 5-HT_3 receptor; (Mouse); Intestinal transit; Prokinetic benzamide

1. Introduction

5-HT_3 receptors seem to mediate physiological and pathological phenomena, such as the induction of gastric motor migrating contractions (Wilmer et al., 1993) and the nausea and vomiting associated with cancer chemotherapy and radiotherapy (Andrews et al., 1988; Andrews and Bhandari, 1993), in humans. In addition, they seem to be involved in the control of intestinal propulsive activity in mammalian species, including humans. It has been reported that ondansetron, a selective 5-HT_3 receptor antagonist, inhibits small intestinal propulsive activity in rats (Brown et al., 1993), and slows colonic transit in healthy humans (Gore et al., 1990). In view of this evidence, activation of 5-HT_3 receptors seems to be one factor that underlies the physiological propulsion of chyme through the intestine.

Gastrointestinal prokinetic benzamides, such as renzapride, zacopride and cisapride, are chemically related to metoclopramide and enhance gastrointestinal propulsion and coordinated motility. Some of these are now being introduced into clinical use for the treatment of gastrointestinal motility disorders (Briejer et al., 1995). It is well known that they all have moderate to high affinity for 5-HT_3 receptors, on which they act as antagonists (Schiavone et al., 1990). Their prokinetic effects on lower intestinal propulsive activity appear now less certain compared with those on gastroduodenal propulsive activity.

In the present study, the role of 5-HT_3 receptors in the regulation of intestinal propulsive activity was investigated in mice by a simple method in which the time taken for excretion of the head of an orally administered non-absorbable marker (whole gut transit time) was measured. We first evaluated the effects of the selective 5-HT_3 receptor antagonists ramosetron (YM060; Miyata et al., 1991a) and ondansetron (Butler et al., 1988). Next, the effects of prokinetic benzamides with 5-HT_3 receptor antagonistic activity, viz. renzapride, zacopride and cisapride,

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were also examined. Further, the effects of ramosetron and ondansetron on upper gastrointestinal transit were assessed to determine whether the inhibition of whole gut propulsion by the 5-HT_3 receptor blockade include that of upper gastrointestinal propulsion or just reflect that of lower intestinal propulsion.

2. Materials and methods

2.1. Animals

Male ICR mice (Japan SLC, Hamamatsu, Japan) weighing 23–32 g were used. Animals were housed under standard controlled environmental conditions, with a 12-h light cycle (08:30–20:30 h). Food and water were available ad libitum. On the day of the experiment, each mouse was transferred to a transparent plastic individual cage (W $100 \times L$ $170 \times H$ 110 mm) at 10:00 h and kept for 1 h before the application of drugs.

2.2. Whole gut transit test

Mice were randomly divided into 5 groups (8 animals per group). Carmine employed as a marker was orally administered to each mouse at 0.3 ml (3 g of carmine in 50 ml of 0.5% methylcellulose). Mice were then returned to the individual cage, which was placed on a white sheet so as to distinguish stools colored by the marker from normal stools. The time taken for excretion of the head of the orally administered marker was measured. The endpoint was taken as the first appearance of one colored (red) pellet, and the appearance of the marker was based on visual observation. The observation was performed for 8 h after the administration of marker. Each mouse was subcutaneously treated with either vehicle (10 ml/kg s.c.) or a single dose of test drug just before administration of marker.

2.3. Upper gastrointestinal transit test

Mice were randomly divided into 5 groups (8 animals per group). After oral administration of 0.3 ml of marker (as used in the whole gut transit test), the mouse was returned to the individual cage. At 20 min after the administration of marker, they were sacrificed by cervical dislocation, the abdomen was opened and the intestine was removed from the pyloric junction to the caecal end. The distance traveled by the head of the marker and the total length of the intestine were measured. Upper gastrointestinal transit was expressed as a percentage of the distance traveled by the head of the marker relative to the total length of the small intestine. Each mouse was subcutaneously treated with either saline (10 ml/kg s.c.) or a single dose of test drug 30 min before the administration of marker. This experimental condition (20-min interval) is considered suitable for evaluating the effects of drugs on upper gastrointestinal propulsive activity.

2.4. Statistical analysis

The results are expressed as the means ± S.E.M. Group data of upper gastrointestinal and whole gut transit were compared by analysis of variance followed by Dunnett's multiple range test. P values less than 0.05 were considered significant.

2.5. Drugs

Ramosetron (YM060) hydrochloride, ondansetron hydrochloride, renzapride hydrochloride, zacopride fumarate were all prepared by Yamanouchi Pharmaceutical (Tsukuba, Japan). Cisapride was extracted and purified from Acenalin Tab. (Janssen-Kyowa, Tokyo, Japan). Clonidine hydrochloride was purchased from Research Biochemicals (Natick, MA, USA), and neostigmine bromide and carmine were purchased from Wako Pure Chemical Industries (Osaka, Japan). Ramosetron, ondansetron, renzapride, zacopride, clonidine and neostigmine were dissolved in physiological saline. Cisapride was dissolved in 0.1% lactic acid. Volume of administration for s.c. treatment was 10 ml/kg. All drug dosages were in terms of the free base.

3. Results

3.1. Whole gut transit test

Administration of 0.3 ml marker (3 g carmine in 50 ml 0.5% methylcellulose) did not cause diarrhea in any mouse. Stools colored by the marker were the same as normal stools in form, but could easily be distinguished from them by their red color. Preliminary experiments showed that the mean time taken for excretion of the head of orally administered marker (whole gut transit time) was around 200 min and stable, being independent of the experimental days. The acetylcholinesterase inhibitor neostigmine, which is reported to stimulate intestinal propulsive activity, shortened the whole gut transit time at 0.003–0.1 mg/kg s.c., whereas the α_2-adrenoceptor agonist clonidine, which is well known to reduce intestinal propulsive activity, prolonged it at 0.01–0.3 mg/kg s.c. in a dose-dependent manner (Fig. 1A,B). These observations confirm that this assay system is appropriate for examination of both the stimulatory and inhibitory effects of drugs on intestinal propulsive activity.

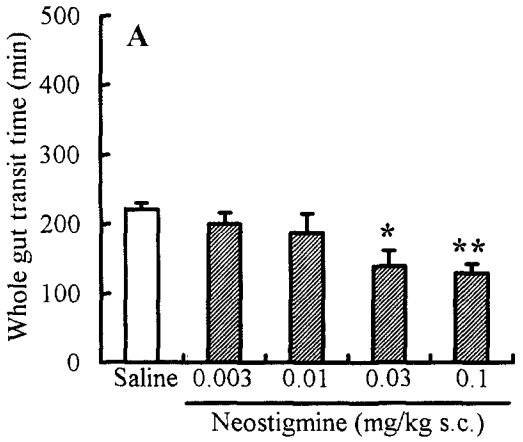
First, we examined the effects of selective 5-HT_3 receptor antagonists. Ramosetron at 0.01–0.3 mg/kg s.c. and ondansetron at 0.1–1 mg/kg s.c. prolonged the whole gut

transit time in a dose-dependent manner. These effects of ramosetron and ondansetron at doses equal to or greater than 0.1 and 1 mg/kg s.c., respectively, were significant compared with that of vehicle (Fig. 2A,B).

Next, we investigated the effects of prokinetic benzamides. Renzapride at 1–10 mg/kg s.c., zacopride at 0.01–0.3 mg/kg s.c. and cisapride at 0.1–3 mg/kg s.c. dose-dependently prolonged the whole gut transit time. These effects of renzapride, zacopride and cisapride at doses equal to or higher than 10, 0.3 and 3 mg/kg s.c., respectively, were significant compared with that of each vehicle (Fig. 3A–C).

3.2. Upper gastrointestinal transit test

Upper gastrointestinal transit over 20 min was approximately 50% in the control (no treatment) study. This result is in accord with those of studies by the standard method employing a charcoal meal as a marker. Ramosetron and ondansetron had no effect on upper gastrointestinal transit at doses up to 1 mg/kg s.c. (Fig. 4A,B).



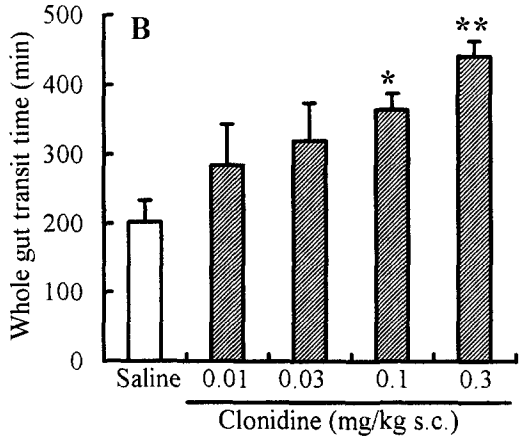
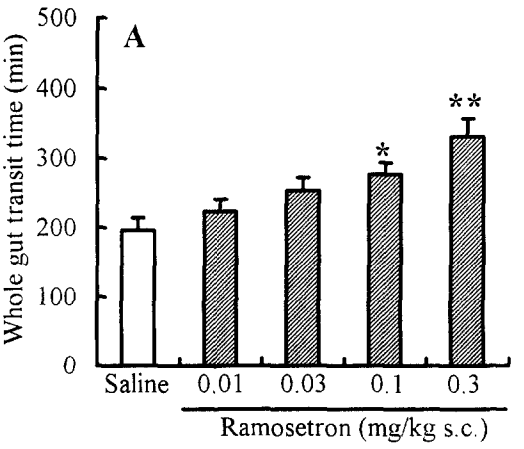


Fig. 1. Effects of neostigmine (A), an acetylcholinesterase inhibitor, and clonidine (B), an α_2-adrenoceptor agonist, on intestinal propulsive activity in ICR mice. The time taken for excretion of the head of an orally administered marker (whole gut transit time) was measured. Test drugs were s.c. administered just before marker administration. Each column and vertical bar represent the mean ± S.E.M. in 8 mice. * $P < 0.05$; ** $P < 0.01$, significant differences from saline treatment.



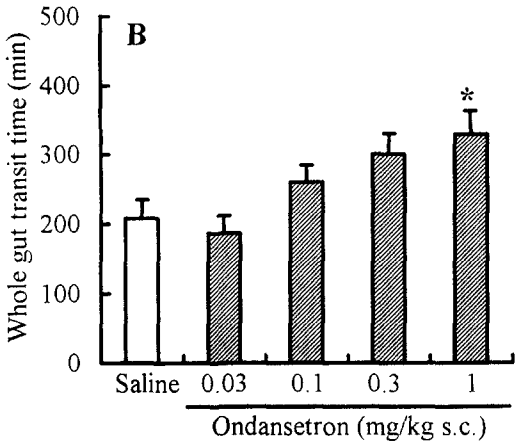


Fig. 2. Effects of selective 5-HT_3 receptor antagonists ramosetron (A) and ondansetron (B) on intestinal propulsive activity in ICR mice. The time taken for excretion of the head of an orally administered marker (whole gut transit time) was measured. Test drugs were s.c. administered just before marker administration. Each column and vertical bar represent the mean ± S.E.M. in 8 mice. * $P < 0.05$; ** $P < 0.01$, significant differences from saline treatment.

4. Discussion

We investigated the role of 5-HT_3 receptors in the control of intestinal propulsive activity in mice by a simple method in which the time taken for excretion of the head of an orally administered non-absorbable marker (whole gut transit time) was measured. Neostigmine (an acetylcholinesterase inhibitor) and clonidine (an α_2-adrenoceptor agonist) are reported to facilitate (Kishibayashi and Karasawa, 1995) and inhibit (Ruwart et al., 1980; Baxter et al., 1987) intestinal propulsive activity, respectively, in rats and humans. We used these two agents to validate whether this method allows detection of the effects of drugs. Re-

sults showed that neostigmine at 0.003–0.1 mg/kg s.c. and clonidine at 0.01–0.3 mg/kg s.c. dose-dependently shortened and prolonged whole gut transit time in mice,

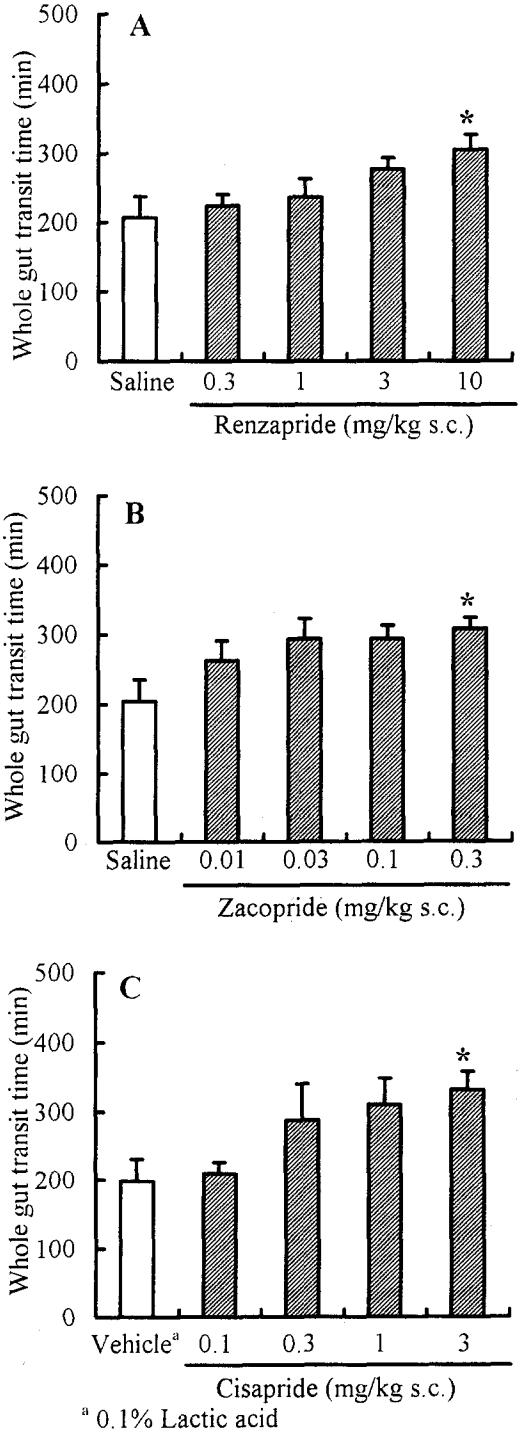


Fig. 3. Effects of prokinetic benzamides renzapride (A), zacopride (B) and cisapride (C) on intestinal propulsive activity in ICR mice. The time taken for excretion of the head of an orally administered marker (whole gut transit time) was measured. Test drugs were s.c. administered just before marker administration. Each column and vertical bar represent the mean ± S.E.M. in 8 mice. * $P < 0.05$, significant difference from vehicle treatment.

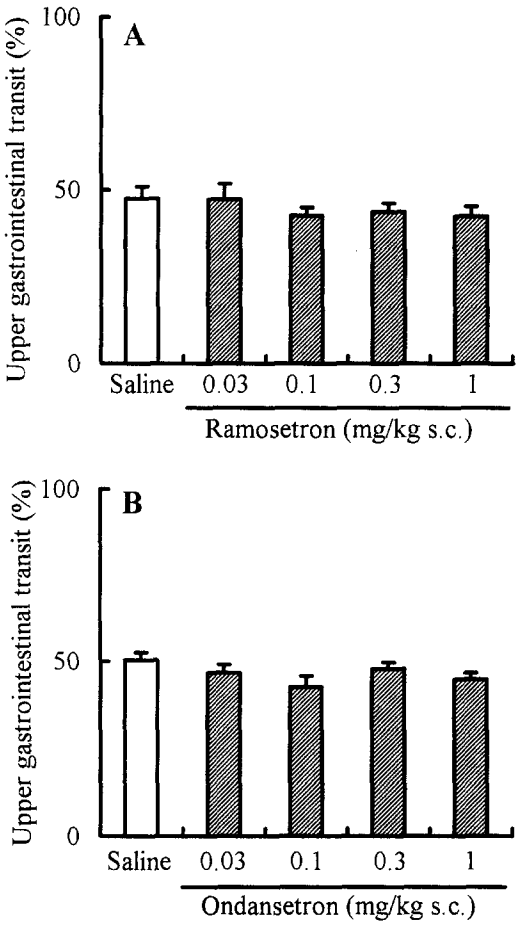


Fig. 4. Effects of selective 5-HT_3 receptor antagonists ramosetron (A) and ondansetron (B) on upper gastrointestinal propulsive activity in ICR mice. Upper gastrointestinal transit was expressed as a % of the distance traveled by an orally administered marker relative to the total length of the small intestine over 20 min after marker administration. Test drugs were s.c. administered 30 min before marker administration. Each column and vertical bar represent the mean ± S.E.M. in 8 mice.

respectively, confirming that this assay system is appropriate for the assessment of the stimulatory and inhibitory effects of test drugs on intestinal propulsive activity. This method requires neither surgical operation nor complicated techniques, and therefore minimizes the effect of artifact and other influence on intestinal propulsive activity in the physiological condition. Furthermore, this method can be applied to small animals, such as mice, valuable when evaluating the effects of a number of compounds on intestinal propulsive activity. We therefore propose that this mouse model is suitable for use in studies on physiological intestinal propulsive activity and in the evaluation of both stimulatory and inhibitory effects of test drugs.

Ramosetron and ondansetron, which are selective 5-HT_3 receptor antagonists, prolonged whole gut transit time, indicating that endogenous 5-HT plays a physiological role in sustained stimulation of intestinal propulsive activity via 5-HT_3 receptors. Because they did not affect upper gastro-

intestinal transit at doses which significantly prolonged whole gut transit time, it is likely that the site at which 5-HT_3 receptor antagonists reduce propulsive activity is the lower intestine (ileum, caecum and colon). In contrast to our results, it has been reported that ondansetron does not inhibit colonic propulsive activity in rats (Kadowaki et al., 1993). Although it cannot be ruled out that this discrepancy is attributable to methodological differences, a species difference in the role of 5-HT_3 receptors in the control of lower intestinal propulsive activity may exist between rats and mice. In healthy humans, ondansetron has been reported to inhibit colonic propulsive activity (Gore et al., 1990) in accord with our results; the role of 5-HT_3 receptors in the physiological control of lower intestinal propulsive activity in humans may therefore be closer to that in mice than that in rats.

All tested prokinetic benzamides, renzapride, zacopride and cisapride, also prolonged whole gut transit time in mice in the present study. Because it has been reported that prokinetic benzamides, including cisapride, enhance gastric emptying (Kato et al., 1990) and fail to affect upper gastrointestinal propulsive activity (Iwanaga et al., 1991) in mice, the site at which prokinetic benzamides inhibit propulsive activity also seems to be the lower intestine. Most prokinetic benzamides share moderate to high affinity for 5-HT_3 receptors, acting as antagonists (Schiavone et al., 1990; Briejer et al., 1995). It is possible that their antagonistic effects at 5-HT_3 receptors inhibit lower intestinal propulsive activity. In rats, renzapride, zacopride, cisapride, ramosetron and ondansetron inhibit the Von Bezold-Jarisch reflex, a well-characterized 5-HT_3 receptor-mediated bradycardiac response, with ED_{50} values of 12.4, 0.4, 2022 (Schiavone et al., 1990), 0.036 and 1.9 μg/kg i.v. (Miyata et al., 1991b), respectively. Renzapride, zacopride, cisapride, ramosetron and ondansetron prolonged the whole gut transit time with the minimal effective doses of 10, 0.3, 3, 0.1 and 1 mg/kg s.c., respectively. The rank order of potency in the Von Bezold-Jarisch reflex test is ramosetron > zacopride > ondansetron > renzapride ≫ cisapride, whereas that in the whole gut transit test is ramosetron > zacopride > ondansetron > cisapride > renzapride. Taken together, the rank order of potency of renzapride, zacopride, ramosetron and ondansetron in the whole gut transit test roughly correlates with that in the Von Bezold-Jarisch reflex test, suggesting that the inhibitory effect of these 4 compounds on intestinal propulsive activity is attributable, at least in part, to 5-HT_3 receptor blockade. However, the potency of cisapride in the whole gut transit test is apparently higher than anticipated from that in the Von Bezold-Jarisch reflex test: the potency of cisapride is approximately 1000-fold weaker than that of ondansetron in the Von Bezold-Jarisch reflex test, but only 3-fold weaker than that of ondansetron in the whole gut transit test. This discrepancy may indicate that the inhibitory effect of cisapride, unlike the case of the other prokinetic benzamides renzapride and zacopride, is due to mechanisms additional to or even other than 5-HT_3 receptor blockade.

Prokinetic benzamides are well known to have 5-HT_4 receptor agonistic in addition to 5-HT_3 receptor antagonistic effect. It is possible that 5-HT_4 receptor agonistic property of prokinetic benzamides contributes to the inhibition of intestinal propulsive activity in the present study and explains the odd rank order of cisapride. Examination of the effect of prokinetic benzamides under the treatment of selective in vivo active 5-HT_4 receptor antagonist is needed to exclude this possibility. However, a selective and in vivo active 5-HT_4 receptor antagonist SB 204070 (Wardle et al., 1994; Hegde et al., 1994) prolonged the whole gut transit time at doses to antagonize the 5-HT_4 receptor (data not shown). It is likely that 5-HT_3 receptor antagonistic but not 5-HT_4 receptor agonistic property of prokinetic benzamides contributes to the inhibition of lower intestinal propulsive activity in mice because selective blockade of 5-HT_4 receptors inhibited gut propulsive activity in mice. Cisapride is known to have high affinity not only for 5-HT_3 and 5-HT_4 receptors but also for 5-HT_{2A}, D_2 and α_1 receptors (Karasawa et al., 1990). The odd rank order of this compound in the present study may be due to its composite interaction with these receptors except 5-HT_4 receptors.

Clinically, prokinetic benzamides are used to treat gastrointestinal dysmotility, and it is well established that these compounds have therapeutic benefits for gastroduodenal dysmotility. However, their prokinetic potential for lower intestinal motor activity seems less certain. The effect of cisapride, the most widely used prokinetic benzamide, on colonic motility in humans is now controversial, and this drug is only moderately effective in humans (Briejer et al., 1995). The present study demonstrates that renzapride and zacopride, possibly via 5-HT_3 receptor blockade inhibit lower intestinal propulsive activity in mice, and may suggest that prokinetic benzamides, which have 5-HT_3 receptor antagonistic activity, are unsuitable for the treatment of impaired lower intestinal propulsive activity. Cisapride also inhibited lower intestinal propulsive activity in mice although this inhibitory effect could not be explained only by its 5-HT_3 receptor antagonistic effect. This result may suggest that cisapride, in contrast to its established therapeutic benefits for gastroduodenal dysmotility, is also not ideal for the treatment of impaired lower intestinal propulsive activity.

In conclusion, we employed a simple method, the whole gut transit test in mice, to investigate intestinal propulsive activity under physiological conditions. Results indicate that endogenous 5-HT plays a physiological role in the sustained stimulation of lower intestinal propulsive activity via 5-HT_3 receptors. The prokinetic benzamides renzapride and zacopride possibly via 5-HT_3 receptor blockade, and cisapride via an as yet unidentified mechanism inhibit lower intestinal propulsive activity. These results suggest that prokinetic benzamides, at least those which have

5-HT_3 receptor antagonistic activity, may, in contrast to their therapeutic benefits for gastroduodenal dysmotility, be unsuitable for the treatment of impaired lower intestinal propulsive activity.

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