J Nat Med (2011) 65:89–94
DOI 10.1007/s11418-010-0463-0

ORIGINAL PAPER

Inhibitory effects of zingerone, a pungent component of *Zingiber officinale* Roscoe, on colonic motility in rats

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Received: 20 May 2010 / Accepted: 2 August 2010 / Published online: 27 August 2010

Abstract Ginger (rhizome of *Zingiber officinale* Roscoe) is an herbal medicine for the treatment of gastrointestinal disorders including constipation and diarrhea. Zingerone is a likely active constituent responsible for the antidiarrheal activity of ginger. The current study was designed to characterize pharmacological actions of zingerone on colonic motility. To evaluate pharmacological effects of zingerone on colonic motility, we used isolated colonic segments from rats, in which mechanical responses were recorded in the longitudinal direction. In addition, we evaluated the effects on colonic motility in vivo by measuring intraluminal pressure changes and expelled fluid volume from the colon in anesthetized rats. Zingerone was applied to the lumen of the colon to allow the drug to access from the mucosal side. Zingerone inhibited spontaneous contractile movements in the isolated colonic segments in a dose-dependent manner. The inhibitory effects of zingerone on colonic movements were not affected by pretreatment with capsazepine, a typical antagonist of transient receptor potential vanilloid 1. In addition, tetrodotoxin, a blocker of voltage-dependent sodium channels on neurons, did not affect the suppression of colonic movements by zingerone, suggesting that zingerone acts on the smooth muscles directly. Zingerone also attenuated colonic motility in vivo without affecting blood pressure and heart rate. The effects were reversible and reproducible. Our findings suggest that zingerone can inhibit colonic motility via direct action on smooth muscles. Zingerone might exert beneficial therapeutic effects on hypermotility-induced diarrhea by abrogating excessive gastrointestinal motility.

Keywords Colon · Diarrhea · Gastrointestinal motility · Ginger · Zingerone

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Introduction

Ginger, the rhizome of *Zingiber officinale* Roscoe, has a long history of medicinal use in Asian countries for the treatment of headaches, nausea, rheumatism, and gastrointestinal disorders [1–4]. In accordance with this, it has been reported that ginger has various pharmacological actions. For example, ginger exerts anti-inflammatory, anticancer, and antiemetic activities as well as therapeutic effects on constipation and diarrhea [3, 5, 6]. Multiple pungent and non-pungent components, such as shogaol, gingerol, and zingerone, are included in ginger [7]. Several lines of evidence indicate that the components are dominantly related to the actions of ginger [1].

Zingerone is one of the pungent components of ginger, which is included in up to 3% of essential oil with gingerol and shogaol [8]. It has been demonstrated that zingerone inhibits enterotoxin-induced fluid secretion in the ileum in mice [9]. Since excessive fluid secretion of gastrointestinal tracts causes diarrhea [10], it can be considered that zingerone is the likely active constituent responsible for the antidiarrheal activity of ginger. In addition to the excessive secretion, abnormal facilitation of gastrointestinal motility would be a cause of diarrhea [11]. In accordance with this,

some herbal medicines possess an antidiarrheal activity, which may be related to inhibition of intestinal muscle motility [12–14]. Interestingly, it has been reported that ginger also has suppressive effects on gut motility. Crude ginger extract inhibits rat ileal motility via inhibition of enteric neural excitatory transmission and smooth muscle mechanical activity in vitro [15]. Ghayur and Gilani [4] reported inhibitory effects of ginger crude extract on high K^+-induced contractions in isolated guinea pig colon. Furthermore, herbal medicines that include ginger extracts inhibit colonic motility in rats [16]. These findings suggest that some constituents of ginger exert beneficial therapeutic effects on hypermotility-induced diarrhea by abrogating excessive gastrointestinal motility.

The current study was designed to corroborate the antidiarrheal activity of zingerone. For this purpose, we focused on possible effects of the compound on colonic motility, because its effects on fluid secretion in the gut have already been reported. First, we examined effects of intraluminal application of zingerone on the motility of isolated colonic segments of rats. Then we recorded in vivo colonic motility. The data obtained show that zingerone has the ability to inhibit colonic motility via direct action on smooth muscles.

Materials and methods

Experimental animals

Male Wistar rats (12–15 weeks of age, 300–500 g) were maintained in plastic cages at 22 ± 2°C with a 12:12-h light–dark cycle. They were given free access to laboratory chow (LABO MR Stock, Nihon-Nosan) and water. The experiments were approved by the Animal Care and Use Committee of Gifu University.

Tissue preparation

Animals were exsanguinated via the carotid arteries after etherization. The abdominal cavity was opened immediately and a 3- to 4-cm-long segment of the distal colon (2 cm advance to the anus) was dissected out and immersed in Tyrode's solution (see below) at room temperature. The intraluminal contents were flushed by using a small cannula filled with Tyrode's solution.

Mechanical recordings

Each segment of distal colon (3–4 cm in length) was mounted in longitudinal orientation in a Magnus apparatus (10 ml in capacity) filled with Tyrode's solution. The solution was continuously bubbled with 95% O_2 + 5% CO_2 gas mixture and maintained at 37°C. The distal end of each segment was tied to organ holders and the proximal end was secured with a silk thread to an isometric force transducer (T7-30-240, Orientec). Spontaneous contractile responses were recorded isometrically. An initial tension of 1.0 g was applied to the colonic preparations and no further mechanical adjustment was made during experimentation. Isometric responses were filtered and amplified with an amplifier (AS1202, NEC) and recorded by using a Power Lab system (model 2/25, AD Instruments).

In vivo preparation

Sedation was achieved with ketamine hydrochloride (50 mg/kg) administered intramuscularly. Anesthesia was then induced with α-chloralose (40 mg/kg) administered intravenously. The femoral artery was cannulated and anesthesia was maintained by intra-arterial infusion of α-chloralose (20–25 mg/kg/h) combined with ketamine hydrochloride (5–7 mg/kg/h) in saline (0.9% NaCl). Blood pressure was also measured from the femoral artery. The colon was cannulated in the region of the colonic flexure and at the anus. The body wall was closed with the oral cannula protruding. The oral cannula was connected to a Marriotte bottle filled with warm saline, and the distal cannula was connected to a pressure transducer, one-way valve, and a fluid outlet. The baseline intraluminal pressure was maintained at 3–5 mmHg by adjusting the height of the Marriotte bottle and the outlet tube. Expelled fluid was collected in a cylinder positioned beneath the fluid outlet. At the completion of experiments, rats were killed by intraperitoneal injection of a lethal dose of sodium pentobarbitone (200 mg/kg) while they were still under anesthesia.

Solutions and drugs

Tyrode's solution consisted of (mM): NaCl 135.9, KCl 2.68, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.41, and glucose 5.55 for colonic preparations. Zingerone was a kind gift from Kanebo Institute. Tetrodotoxin, capsazepine, α-chloralose, and ketamine hydrochloride were obtained from Wako, Sigma, Nakalai Tesque, and Daiichi-Sankyo, respectively. Zingerone was dissolved in ethanol. Capsazepine was dissolved in dimethylsulfoxide. Tetrodotoxin was dissolved in citrate solution. The highest concentration of vehicles for the drugs alone had no effect on the basal tone and spontaneous activity in colonic preparations. Zingerone was applied intraluminally using silicon tubes and plastic syringes. Tetrodotoxin and capsazepine were applied in the Magnus tube. The concentrations of drugs given were final concentrations in Tyrode's solution or saline. α-Chloralose was dissolved in ethanol and solubilized with 10% 2-hydroxypropyl-β-cyclodextrin (Wako)

and then made up to an isotonic solution with NaCl for infusion.

Statistical analysis

Data are presented as means ± standard deviation (SD); *n* indicates the number of separate preparations. Spontaneous contractile activities of the colonic segments were expressed as the area under the curve (AUC). The significance of differences between mean values was determined by one-way analysis of variance followed by Dunnett's test. A *P* value less than 0.05 denotes the presence of a statistically significant difference.

Results

Effects of intraluminal application of zingerone on spontaneous movements of longitudinal smooth muscles in the rat distal colon in vitro

When tension was recorded in a longitudinal direction, isolated preparations of the rat distal colon exhibited rhythmic and cyclic movements even in the absence of electrical or chemical stimulations (Fig. 1a). The amplitude and frequency of the spontaneous contractions were sustained stably for at least 1 h after mounting the preparations. Intraluminal application of zingerone (30 and 50 mM) significantly inhibited the colonic spontaneous motility (Fig. 1a, b).

Effects of capsazepine and tetrodotoxin on zingerone-induced inhibition of colonic spontaneous movements

To determine whether inhibitory effects of zingerone on colonic spontaneous motility are mediated via neural pathways, we treated the preparations with capsazepine, a typical antagonist of transient receptor potential vanilloid 1 (TRPV1), or tetrodotoxin, a blocker of voltage-dependent sodium channels on neurons. Treatment of the colonic preparation with capsazepine (100 μM) itself did not affect the basal tone and spontaneous activity in colonic preparations (Fig. 2a). On the other hand, application of tetrodotoxin (1 μM) itself significantly enhanced the spontaneous movements (Fig. 3a). Regardless of their differential effects on the spontaneous movements, neither capsazepine nor tetrodotoxin blocked zingerone-induced inhibition of the spontaneous activity in colonic segments (Figs. 2, 3).

Effects of intraluminal application of zingerone on colonic motility in vivo

We then examined the effects of zingerone on colonic motility in vivo. Under the condition in which intraluminal pressure of the colon was maintained at 3–5 mmHg, the rat distal colon exhibited periodic rises in intraluminal pressure accompanied by fluid output through the anal cannula. Intraluminal application of zingerone (10 mg/kg) decreased both the amplitude of intraluminal pressure changes (Fig. 4a) and the fluid output (control: 2.8 ± 0.8 vs. application of zingerone: 0.8 ± 0.2 ml/10 min, $n = 4$) associated with colonic motility. After washing out intraluminal contents, the colonic motility was recovered (Fig. 4b). The recovered motility was re-blocked by a second application of zingerone (Fig. 4c). The blood pressure and heart rate were not affected by intraluminal application of zingerone (data not shown).

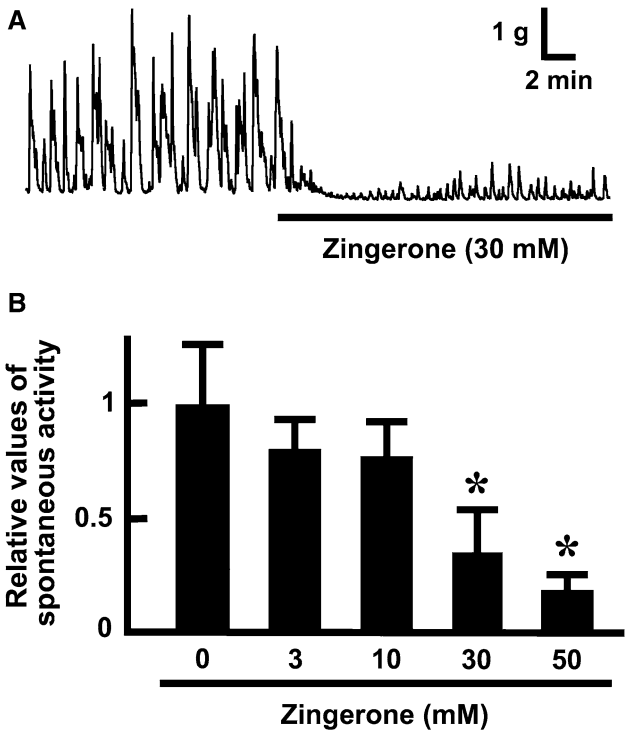

Fig. 1 Effects of zingerone on spontaneous phasic contractions in the rat isolated distal colon. **a** A representative tracing of the colonic spontaneous contractile responses before and after intraluminal application of zingerone (30 mM) is shown. Mechanical responses were recorded isometrically in the longitudinal direction. **b** The inhibitory effects of zingerone (3, 10, 30, and 50 mM) on the colonic spontaneous contractile responses are summarized ($n = 5$). The values of contractile responses are expressed as the area under the curve (AUC) and normalized as ratios of control. *Each bar* represents the mean of data ± SD. *$p < 0.05$, compared to the control

Discussion

In the present study, we examined the pharmacological effects of zingerone, a pungent component of ginger, on colonic motility in rats. Our major findings are: (1) intraluminal application of zingerone inhibited spontaneous

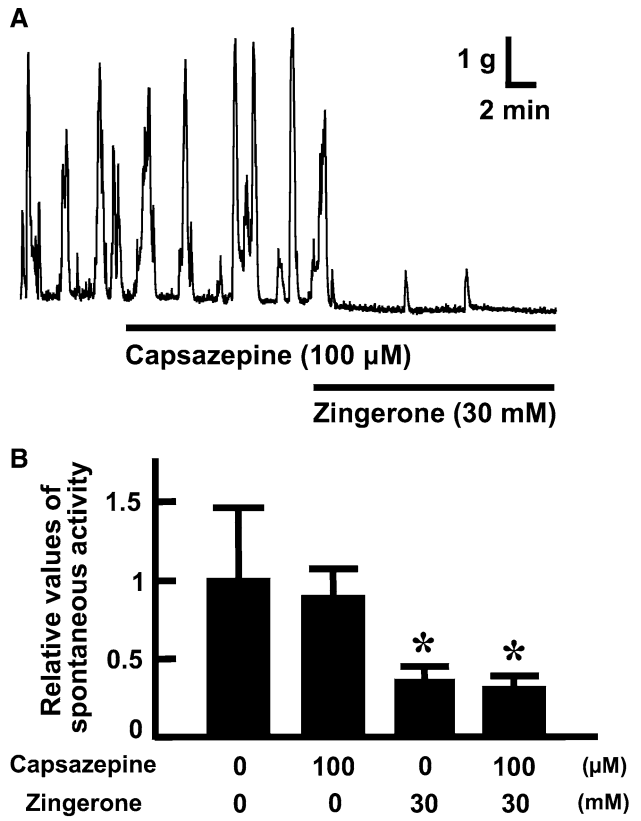

Fig. 2 Effects of capsazepine on zingerone-induced inhibition of colonic spontaneous contractions. **a** A representative tracing demonstrating the inhibitory effects of zingerone (30 mM) on spontaneous contractile responses in the presence of capsazepine (100 μM) is shown. **b** The effects of capsazepine on zingerone-induced inhibition of the colonic spontaneous contractions are summarized ($n = 4$). The values of contractile responses are expressed as AUC and normalized as ratios of control. *Each bar* represents the mean of data ± SD. *$p < 0.05$, compared to the control

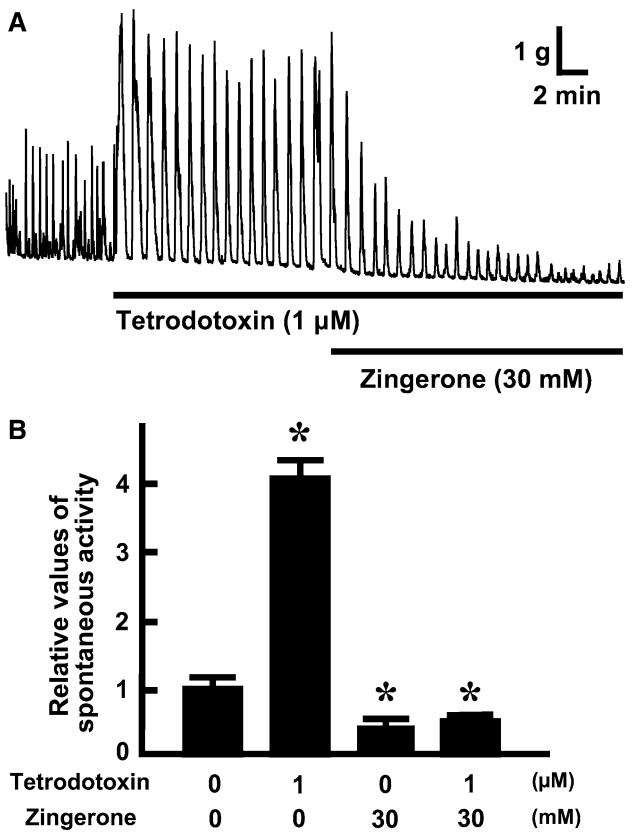

Fig. 3 Effects of tetrodotoxin on zingerone-induced inhibition of colonic spontaneous contractions. **a** A representative tracing demonstrating the inhibitory effects of zingerone (30 mM) on spontaneous contractile responses in the presence of tetrodotoxin (1 μM) is shown. **b** The effects of tetrodotoxin on zingerone-induced inhibition of the colonic spontaneous contractions are summarized ($n = 4$). The values of contractile responses are expressed as AUC and normalized as ratios of control. *Each bar* represents the mean of data ± SD. *$p < 0.05$, compared to the control

contractions in isolated colonic segments, (2) zingerone also attenuated colonic motility in vivo, and (3) the inhibitory effects of zingerone on the colonic movements were not affected by pretreatment with capsazepine or tetrodotoxin for blocking neural components. These findings suggest that zingerone has inhibitory effects on colonic motility via direct action on smooth muscles.

In experiments using isolated intestinal segments, drugs are usually applied to the organ bath. This naturally results in a preferential access of drugs from the serosal side of the segments. We considered that such a method for application of the drug is not appropriate in our experiment because we wanted to examine the effects of a food ingredient on intestinal motility. We therefore applied zingerone to the mucosal side of the colon segments. The spontaneous contractile movements in the isolated colonic segments were inhibited by intraluminal application of zingerone in a dose-dependent manner. It has been shown that some of pungent compounds can activate sensory nerves expressing TRPV1 in the gut [8, 17, 18]. The inhibition of colonic motility by intraluminal application of zingerone can be explained by assuming that zingerone activates sensory nerves and that the activated sensory nerves subsequently trigger on inhibitory neuronal circuit in the enteric nervous system, promoting inhibition of smooth muscle contractions. In support of this assumption, it has been demonstrated in an experiment using the patch-clamp technique that zingerone evokes opening of TRPV1 [19]. Alternatively, it is possible that zingerone exerts a direct inhibitory effect on smooth muscle contractions. To validate these possibilities, pharmacological experiments were carried out.

To clarify whether an activation of TRPV1 on primary afferents is involved in the inhibition of colonic motility by zingerone, we utilized capsazepine, a typical antagonist of TRPV1. If zingerone fails to exert its inhibitory effects in the presence of the antagonist, the involvement of TRPV1

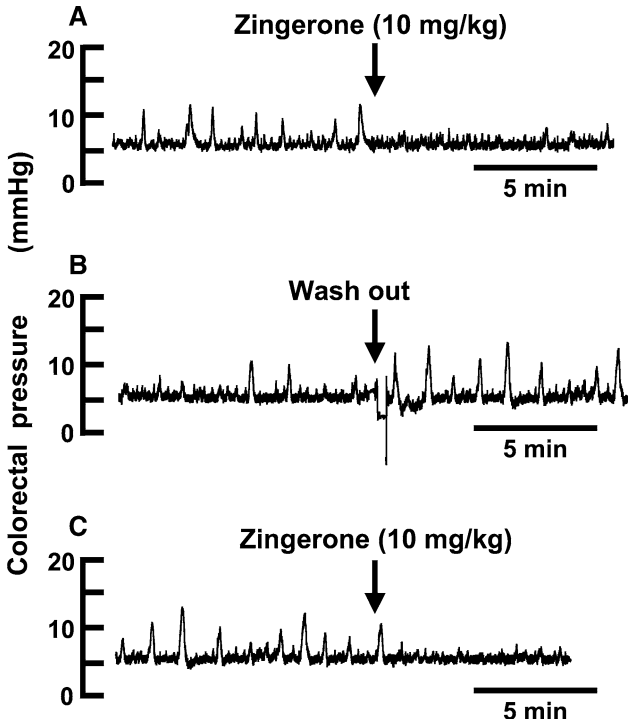

Fig. 4 Inhibitory effects of zingerone on colonic motility in vivo. Intraluminal pressure changes in the colorectum were recorded in vivo. Intraluminal application of zingerone (10 mg/kg) reduced amplitude of the pressure changes (**a**). After washing out intraluminal contents, colonic motility was recovered (**b**). The recovered motility was re-blocked by second application of zingerone (**c**). Similar results were obtained in four independent experiments

would be proven. However, pretreatment with capsazepine did not affect the inhibitory action of zingerone on colonic motility. It is thus rational to assume that the sensory nerves expressing TRPV1 play a minor, if any, role in the inhibitory action of zingerone. It should be noted, however, that this assumption does not necessarily rule out the possible involvement of TRPV1-expressing nerves in other actions of zingerone. For instance, involvement of the nerves in inhibitory effects of zingerone on ileal fluid secretion remain to be determined [9].

The involvement of neural pathways in the inhibitory action of zingerone on colonic motility was further examined by using tetrodotoxin. Since tetrodotoxin is a blocker of voltage-dependent sodium channels, it can prevent neural excitation effectively without affecting excitation of smooth muscles. It has been shown that gut motility is enhanced after application of the blocker [20, 21], also see Fig. 3a. This phenomenon appears to be paradoxical because tetrodotoxin is a blocker, not enhancer, of neural excitation. In general, it is thought that inhibitory neural components are constitutively operated under the basal condition and thereby total inhibition of neural excitation brings about enhancement, not reduction, of gut motility. From the standpoint of pharmacological experiments, the enhancement would provide reliable evidence to show an efficient block of neural activity by tetrodotoxin. Therefore, the fact that zingerone inhibited colonic motility even after enhancing spontaneous motility by tetrodotoxin shows that the inhibition of colonic motility by zingerone is not due to its action on the enteric nervous system but due to a direct action on smooth muscles. In accordance with this, direct inhibitory effects on smooth muscles have been reported for some pungent compounds, including capsaicin and piperine [22–26]. The direct actions on smooth muscles have been shown to be independent of TRPV1 [24–27], being consistent with the fact that zingerone-induced inhibition of colonic motility was resistant to a TRPV1 antagonist.

Zingerone exerts its inhibitory effect on colonic motility at concentrations above 10 mM. Considering that capsaicin is usually used at submicromolar concentrations [8, 28], the concentrations of zingerone seem to be rather high from a pharmacological point of view. However, the concentrations used in the present study would be reasonable, because zingerone is a much less potent analog of capsaicin [8, 19, 29]. For instance, 30 mM of zingerone is necessary to mimic inward currents induced by 1 μM of capsaicin in rat trigeminal ganglion cells [29]. Importantly, in experiments using isolated cells or cultured cells, zingerone up to 100 mM does not cause non-specific damage to the cells [19, 29]. Moreover, the relatively high concentration would also be related to the method of application of zingerone. Since we applied zingerone from the mucosal side, it is expected that the concentration of the drug is diluted before reaching smooth muscles. In fact, lower concentrations of zingerone (e.g., 100 μM) in the serosal side were sufficient to inhibit of colonic motility (our unpublished observation). Therefore, the actions of zingerone on gut smooth muscle may not be due to a non-specific disruption of smooth muscle function.

Zingerone suppressed colonic motility in vivo, confirming the inhibitory effect found in the experiments using isolated colonic segments. Since intracolonic application of zingerone did not affect either blood pressure or heart rate, the effects of zingerone on colonic motility may be locally generated. This suggests that the doses being effective in suppressing colonic contractions do not have unexpected side effects, at least in the cardiovascular system. Thus, zingerone seems to be beneficial for patients with colonic disorder related to hyperexcitability of smooth muscles. In support of this notion, it has been reported that some antidiarrheal drugs, such as loperamide and wood creosote, possess inhibitory effects on gut motility in addition to their modulatory effects on fluid absorption/secretion [30–34]. These agents can act directly on intestinal smooth muscles [35, 36], being consistent with our results. More importantly, the inhibitory effects of zingerone on colonic

motility can be cancelled out quickly by removing (washing out) the compound from the colon. Furthermore, the action of zingerone can be reproduced repetitively. Taken together, the results provide evidence that zingerone is useful as a therapeutic agent for diarrhea.

In conclusion, we characterized the pharmacological actions of zingerone on colonic motility in rats. Our findings suggest that zingerone can inhibit colonic motility via direct action on smooth muscles. Considering that zingerone has the ability to reduce fluid secretion, zingerone will be useful for treating diarrhea and other gastrointestinal disorders.

Acknowledgments We are grateful to Kanebo Institute for the kind gift of zingerone. This work was supported in part by Grants-In-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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