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• *BRIEF REPORTS* •

# Inhibitory effect of fluvastatin on ileal ulcer formation in rats induced by nonsteroidal antiinflammatory drug

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## Abstract

**AIM:** Nonsteroidal anti-inflammatory drugs (NSAIDs) cause gastrointestinal damage as one of their side effects in humans and experimental animals. Lipid peroxidation plays an important role in NSAID-induced ulceration. The aim of this study was to investigate the inhibitory effect of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors on the ulceration in small intestines of rats.

**METHODS:** The effects of three HMG-CoA reductase inhibitors, fluvastatin, pravastatin and atorvastatin on ileal ulcer formation in 5-bromo-2-(4-fluorophenyl)-3-(4-methylsulfonylphenyl) thiophene (BFMeT)-treated rats were examined. Antioxidative activity of the inhibitors was measured by a redox-linked colorimetric method.

**RESULTS:** Fluvastatin, which was reported to have antioxidative activity, repressed the ileal ulcer formation in rats treated with BFMeT an NSAIDs. However, the other HMG-CoA reductase inhibitors (pravastatin and atorvastatin) did not repress the ileal ulcer formation. Among these HMG-CoA reductase inhibitors, fluvastatin showed a significantly stronger reducing power than the others (pravastatin, atorvastatin).

**CONCLUSION:** Fluvastatin having the antioxidaitive activity suppresses ulcer formation in rats induced by NSAIDs.



**Key words:** Ileal ulcer; Fluvastatin; HMG-CoA reductase inhibitors; Nonsteroidal antiinflammatory drug

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## INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs in clinical fields, and several new NSAIDs have recently been developed. However, gastrointestinal ulcers are induced by NSAIDs[1-5]. Allison *et al*[6] reported that small intestinal ulceration occurred in 8.4% of users of NSAIDs but in only 0.6% of non-users. It has also been reported that several patients who were long-term users of NSAIDs died of perforation of small intestinal ulcers[7,8]. These reports suggest that patients who take NSAIDs have an increased risk of ulceration in the small intestine and that small intestinal ulcers can cause life-threatening complications.

Mechanisms of NSAID-induced ulceration have been studied using rat models[4,9,10] and have been reviewed[3,11-13]. Lipid peroxidation mediated by oxygen radicals has been shown to play a crucial role in induction of gastric mucosal damage by NSAIDs, and antioxidants such as ascorbic acid have been shown to attenuate the damage[14-16]. It has been reported that 5-bromo-2-(4-fluorophenyl)-3-(4-methylsulfonylphenyl) thiophene (BFMeT), a non-acidic NSAID[17], induced small intestinal ulcers and that generation of thiobarbituric acid (TBA)-reactive substances, an index of lipid peroxidation, significantly increased in ileal mucosa of rats treated with BFMeT[18]. Some antioxidants, especially ascorbic acid, could repress the ileal ulcer formation in rats treated with BFMeT[19]. It is therefore thought that lipid peroxidation plays an important role in the pathogenesis of gastrointestinal mucosal lesions induced by BFMeT.

In an aging society, many elderly people suffer from more than one disease, and they often use hypercholesterolemic drugs, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, together with NSAIDs. Hypercholesterolemia causes atherosclerosis, which is one of the main causes of cardiovascular diseases. Atherosclerosis has recently become recognized as an inflammatory disease on the basis of results of a study showing that baseline plasma C-reactive protein concentration, a marker for systemic inflammation, was higher in atherosclerosis patients who experienced cardiovascular events than in patients who did not experience any cardiovascular events[20]. Moreover, Ridker *et al*[21] reported that the use of aspirin, an NSAID, reduced the risk of the first occurrence of myocardial infarction in patients with high baseline C-reactive protein concentrations. Therefore, hypercholesterolemic patients

who take HMG-CoA reductase inhibitors could be given NSAIDs. The interaction of NSAIDs and HMG-CoA reductase inhibitors is an important issue. If HMG-CoA reductase inhibitors having antioxidative activity are prescribed for NSAID users, gastrointestinal damage induced by NSAIDs will be reduced. Fluvastatin, which was tested in this study, is a new HMG-CoA reductase inhibitor that has antioxidative activity[5,22]. In this study, we investigated the effect of three HMG-CoA reductase inhibitors (fluvastatin, pravastatin and atorvastatin) on BFMeT-induced ileal ulcer formation in small intestines of rats and compared their antioxidative activities.

## MATERIALS AND METHODS

### Chemicals

BFMeT[17] was obtained from Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). Fluvastatin was kindly supplied from Tanabe Seiyaku Co., Ltd. (Osaka, Japan). Pravastatin and atorvastatin were purchased from Sankyo Co., Ltd. (Osaka, Japan) and Yamanouchi Phrmaceutical Co., Ltd. (Osaka, Japan), respectively. Other reagents, all of reagent grade or higher, were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) or Sigma Chemical Co., (St. Louis, MO).

### Animal treatment

Five-week-old male Wistar rats (100-120 g) obtained from Clea Japan, Inc. (Tokyo) were adapted to laboratory conditions with free access to rat pelleted diet (MF, Oriental Yeast Co., Ltd., Tokyo) and tap water. Rats were housed in plastic cages in a room environmentally controlled at a temperature of 23±2 ℃, humidity of 55±10% and 13-h light/11-h dark cycle. HMG-CoA reductase inhibitors were suspended in 5% gum arabic solution, and 5 or 25 mg/kg of each inhibitor was administered by gavage once a day throughout the experimental period. BFMeT suspended in 5% gum arabic solution was administered at a dose of 1 000 mg/kg of body weight in a single infusion on day 4 by gastric gavage at 13:00 after an 18-h fast as described previously [10]. HMG-CoA reductase inhibitors were administered 4 h after the treatment with BFMeT. The rats were sacrificed 72 h after the administration of BFMeT by cervical dislocation under anaesthesia with diethyl ether, and their gastrointestinal tracts were cut open longitudinally and carefully examined for ulcer formation macroscopically. The ulcer index was calculated as the percentage of total length of the longer diameters of ulcers in the whole length of the small intestine. All animal procedures complied with animal care guidelines of the Institute of Animal Experimentation, School of Medicine, The University of Tokushima.

### Measurement of antioxidative activity

Antioxidative activities of HMG-CoA reductase inhibitors were determined by a redox-linked colorimetric method using iron (Fe (III)) as an easily reduced oxidant in stoichiometric excess[23]. Antioxidants could reduce Fe (III) to Fe (II), which subsequently reacted with 1,10-phenanthroline to form a colored complex[12,24,25]. The intensity of the absorbance at 510 nm reflected the reducing power as antioxidative activity. Each HMG-CoA reductase inhibitor (10 μmol/L) and 0.01 mol/L Fe (III) chloride were subsequently mixed with 0.5 mol/L acetic acid and 0.05 mol/L 1, 10-phenanthroline and incubated for 30 min at room temperature. The colored complex Fe (II)-1, 10-phenanthroline was measured at absorbance of 510 nm (λmax). Gallic acid was used as a positive control.

### Statistical analysis

The significance of differences in the ulcer index and length of the small intestine of two groups and absorbance at 510 nm as the reducing power of HMG-CoA reductase inhibitors were tested by one-way analysis of variance (ANOVA).

## RESULTS

The rats that were administered BFMeT at a dose of 1 000 mg/kg of body weight had ulcers in the small intestine, and the degree of intestinal mucosal damage was the same as that reported previously[18,19]. The rats that were treated with a solvent had no ulcers, and the mean length of the small intestine was 104.8±4.2 cm. Among the tested HMG-CoA reductase inhibitors, fluvastatin showed a repressive effect on the ulceration in small intestines of rats treated with BFMeT (Table 1). The lengths of small intestines in the fluvastatin-treated group were almost the same as those in the non-treated rats. Pravastatin and atorvastatin, however, had no repressive effect. The rats that were treated with a solvent and HMG-CoA reductase inhibitors at doses of 5 and 25 mg/kg had no ulcer formation (data not shown).

**Table 1** Effect of HMG-CoA reductase inhibitors on BFMeT-induced ulcer formation in rat small intestines(mean±SD)

| HMG-CoA reductase inhibitor | Number of rats | Total length of ulcers | Length of the small intestine | Ulcer index (%) |
|---|---|---|---|---|
| None (control) | 7 | 7.1±2.84 | 78.8±10.5 | 9.2±4.10 |
| Fluvastatin 5 mg/kg | 5 | 6.0±3.71 | 83.4±4.6[a] | 7.0±4.13 |
| Fluvastatin 25 mg/kg | 5 | 2.4±0.77[a] | 97.4±12.5[a] | 2.6±1.00[a] |
| Pravastatin 5 mg/kg | 5 | 5.4±2.38 | 87.9±15.9 | 6.6±3.76 |
| Pravastain 25 mg/kg | 4 | 8.0±2.88 | 87.9±11.4 | 9.6±4.47 |
| Atorvastatin 5 mg/kg | 5 | 11.3±3.28[a] | 82.8±14.2 | 14.0±4.79 |
| Atorvastatin 25 mg/kg | 5 | 6.8±4.56 | 85.9±17.2 | 9.3±7.48 |

All rats were administered BFMeT at a dose of 1 000 mg/kg of body weight. Ulcer index was calculated as the percentage of total length of ulcers in the whole length of the small intestine. There was no ulcer formation in small intestines of rats treated with a solvent only and the length of the small intestine was 104.8±4.2 cm. Significantly different from the control group at [a]$P<0.05$ (ANOVA).

The reducing power, as the antioxidative activity of HMG-CoA reductase inhibitors, is shown in Table 2. The reducing power of fluvastatin was significantly stronger than that of the other HMG-CoA reductase inhibitors ($P<0.0001$). Pravastatin showed no antioxidative activity.

**Table 2** Reducing power of HMG-CoA reductase inhibitors(mean±SD)

| HMG-CoA reductase inhibitor | Dose (mmol/L) | Absorbance at 510 nm (as the reducing power) |
|---|---|---|
| Solvent only | 0 | 0.182±0.019 |
| Fluvastatin | 0.01 | 0.545±0.016[2] |
| Pravastatin | 0.01 | 0.182±0.021 |
| Atorvastatin | 0.01 | 0.308±0.016[2] |
| Gallic acid | 0.005 | 0.709±0.010[1] |

The intensity of the absorbance at 510 nm reflected antioxidative activity as the reducing power. [1]Gallic acid was not a HMG-CoA reductase inhibitor and used as a positive control of this test. [2]Significantly different from the solvent at $P<0.0001$ (ANOVA).

## DISCUSSION

In the small intestine, NSAIDs could increase intestinal permeability and enhance exposure of the mucosa to luminal aggressive factors such as bacteria or their degradation products, resulting in an increase in recruitment of neutrophils and their activation[1,3,9,11-13]. Reactive oxygen species produced by activated neutrophils could play important roles in NSAID-induced formation of ulcers[26,27]. As shown in Table 1, atorvastatin did not repress ileal ulcer formation. Although atorvastatin showed a reducing power as could be seen in Table 2, there are no reports of atorvastatin showing inhibition of neutrophil-dependent $O_2^-$ production. Pravastatin has been reported to repress superoxide generation in neutrophils[28], but it showed no reducing power in the present study (Table 2). Fluvastatin has been reported to suppress the generation of superoxide anions from neutrophils and to have a strong antioxidative activity[29-31]. These results suggest that the repressive effect of fluvastatin on ulcer formation is due to its antioxidative activity and that drugs that have an antioxidative activity could repress ileal ulcer formation induced by NSAIDs.

The effects of combinations of drugs on human health have recently become important issues because many elderly patients now take more than one drugs at the same time. Naturally, patients taking NSAIDs should be given a drug or should consume food that has an antioxidative activity and represses ulcer formation induced by NSAIDs. The results of this study indicate that drugs that reduce the side effects of NSAIDs should be selected for patients taking NSAIDs who require treatment with other drugs.

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