Effects of 2-(*E*-2-Decenoylamino)ethyl 2-(Cyclohexylethyl) Sulfide on Various Ulcer Models in Rats

Isao KOHDA,*^a Hitoshi NAGAI,^a Masakazu IWAI,^a Masahiro WATANABE,^a Kazumasa YOKOYAMA,^a Kazutake TSUJIKAWA^b and Tsutomu MIMURA^b

Central Research Laboratories, The Green Cross Corporation,^a Shodai Ohtani 2-1180-1, Hirakata, Osaka 573, Japan and Faculty of Pharmaceutical Sciences, Osaka University,^b Yamadaoka 1-6, Suita, Osaka 565, Japan. Received December 17, 1990

The effects of 2-(*E*-2-decenoylamino)ethyl 2-(cyclohexylethyl) sulfide (compd. III-1a) on various experimental ulcers were investigated. The oral administration of compd. III-1a at doses ranging from 30 to 300 mg/kg inhibited the acute gastric ulcerations induced by ethanol, HCl·aspirin and indomethacin in rats. Compound III-1a significantly inhibited the water immersion stress-induced gastric ulcer at doses of 3 mg/kg, *p.o.* The anti-ulcer activity of plaunotol as a reference drug was equivalent on an ethanol-induced ulcer to that of compd. III-1a, but weaker on HCl·aspirin, indomethacin and stress-induced ulcers than that of compd. III-1a. On indomethacin-produced gastric antral ulcer, compd. III-1a showed the same significant inhibitory activity as spizofurone did at a dose of 100 mg/kg, *p.o.* Compound III-1a also inhibited hemorrhagic shock-, diethyldithiocarbamic acid (DDC)- and platelet activating factor (PAF)-induced ulcers dose-dependently. Plaunotol only showed significant inhibitory activity on PAF-induced ulcer in these three ulcer models. The consecutive administration of compd. III-1a (100 mg/kg, *p.o.*) twice a day significantly accelerated the healing of an acetic acid-induced ulcer and that of plaunotol (200 mg/kg, *p.o.*) showed the same activity. Moreover, orally administered compd. III-1a at a dose of 100 mg/kg decreased the gastric acid secretion in pylorus-ligated rats. The results in the present study suggest that compd. III-1a has the dual action on ulcer formation.

Keywords 2-(*E*-2-decenoylamino)ethyl 2-(cyclohexylethyl) sulfide; ethanol-induced ulcer; aspirin-induced ulcer; indomethacin-induced ulcer; water immersion stress-induced ulcer; indomethacin-produced gastric antral ulcer; hemorrhagic shock-induced ulcer; PAF-induced ulcer; acetic acid-induced ulcer; pylorus ligation gastric secretion

Previously, we reported that among the derivatives of 2-(*E*-2-alkenoylamino)ethyl alkyl sulfide, 2-(*E*-2-decenoylamino)ethyl 2-(cyclohexylethyl) sulfide (compd. III-1a) showed the strongest inhibitory activity on water immersion stress-induced ulcer and low toxicity.¹⁾

In this work, the pharmacological profile of this compound as an anti-ulcer agent was studied by using various ulcer models.

Experimental

Materials 2-(*E*-2-Decenoylamino)ethyl 2-(cyclohexylethyl) sulfide was synthesized as described previously.¹⁾ HCO-60 was obtained from Nikko Chemicals. Acetylsalicylic acid (aspirin) and diethyldithiocarbamic acid sodium salt, trihydrate (DDC) were obtained from Nakarai tesque. Indomethacin and platelet activating factor (PAF) were obtained from Sigma. Plaunotol (Kelnac®, Sankyo) and spizofurone (Maon®, Takeda) were used as a reference drugs.

Experimental Animal Male Wistar rats weighing 180 g (6 weeks)—250 g (7 weeks), fasted for 24 h, were purchased from Charles River Japan Inc.

Experimental Gastric Ulcer Models in Rats i) Ethanol-Induced Ulcer: This ulcer model was induced according to the method of Kuwata *et al.*²⁾ The rats were given perorally 1 ml/rat of 70% ethanol. Each sample was suspended with 10% HCO-60 in saline and administered orally, 0.5 h before the ethanol administration. The animals were sacrificed 1 h later and their ulcer indices were evaluated.

ii) HCl·Aspirin-Induced Ulcer: According to the method of Guth *et al.*,³⁾ the rats were given perorally 150 mg/kg of aspirin, suspended with 5% gum arabica in 150 mm HCl. Each sample was suspended with 10% HCO-60 in saline and administered orally, 0.5 h before the aspirin administration. The animals were sacrificed 1 h later and their ulcer indices were evaluated.

iii) Indomethacin-Induced Ulcer: By the method of Urusidani *et al.*,⁴⁾ the rats were given subcutaneously 30 mg/kg of indomethacin. Each sample was suspended with 10% HCO-60 in saline and administered orally, immediately before the indomethacin administration. The animals were sacrificed 7 h later and their ulcer indices were evaluated.

iv) Water Immersion Stress-Induced Ulcer: The rats were subjected to stress following the method of Takagi and Okabe,⁵⁾ in which animals were immobilized in a stress cage and immersed vertically in a water bath at 22±1°C to the level of the xiphoid process. Each sample was suspended with 10% HCO-60 in saline and administered orally, immediately before the stress treatment. The animals were sacrificed 7 h after being subjected to the stress and their ulcer indices were evaluated.

v) Indomethacin-Produced Gastric Antral Ulcer: This ulcer model was induced according to the method of Satoh *et al.*⁶⁾ Indomethacin (30 mg/kg) was given subcutaneously after the refeeding. Each sample was suspended with 10% HCO-60 in saline and administered orally, 0.5 h before the refeeding. One milliliter of a 1% Evans blue solution was injected into each rat *via* the tail vein immediately before the animals were sacrificed 8 h later and their ulcer indices were evaluated.

vi) Hemorrhagic Shock-Induced Ulcer: This ulcer model was induced according to the method of Ito *et al.*,⁷⁾ with slight modification as followed. The rats were anesthetized with urethane. The blood which corresponded to 2% of the rat's body weight, was withdrawn from the carotid artery over a 5 min period into a syringe containing heparin. Half an hour after the hemorrhagic shock, the shed blood was returned into the carotid artery over 5 min. One milliliter of a 1% Evans blue solution was injected into each rat *via* the tail vein immediately before the animals were sacrificed 0.5 h after the reperfusion and their ulcer indices were evaluated. Each sample was suspended with 10% HCO-60 in saline and administered orally, 0.5 h before the hemorrhagic shock.

vii) DDC-Induced Ulcer: This ulcer model was induced according to the method of Ogino *et al.*⁸⁾ The rats were given subcutaneously 1000 mg/kg of DDC. Each sample was suspended with 10% HCO-60 in saline and administered orally, 0.5 h before the DDC administration. The animals were sacrificed 7 h later and their ulcer indices were evaluated.

viii) PAF-Induced Ulcer: This ulcer model was induced according to the method of Satoh *et al.*⁹⁾ The rats were given intravenously 8 µg/kg of PAF. Each sample was suspended with 10% HCO-60 in saline and administered orally, 0.5 h before the PAF administration. The animals were sacrificed 1 h later and their ulcer indices were evaluated.

ix) Acetic Acid-Induced Ulcer: The experiment was carried out according to the methods of Takagi *et al.*¹⁰⁾ and Okabe *et al.*¹¹⁾ The rats were anesthetized with ether. The abdomen was incised and the stomach was exposed. 0.02 ml of 20% acetic acid was injected into the submucosal layer of the antral-oxyntic border on the anterior wall, then the abdomen was closed. Thereafter, the animals were fed normally and each sample was mixed with soybean oil and administered orally, twice a day for 14 d from the fifth day after the operation. The animals were sacrificed 16 h after the final administration of each sample and their ulcer indices were evaluated.

Evaluation of Gastric Ulcer Models Ulcerated stomachs were removed, inflated with 10 ml of saline and immersed in 1% formalin solution for 5 min. The stomach was incised along the greater curvature and examined for gastric lesions. The total length (mm) of all lesions in the glandular portion of the stomach was used as an ulcer index on ethanol-, HCl·aspirin-, indomethacin-, water immersion stress-, hemorrhagic shock- and DDC-induced ulcer models. The total diameter (mm) of all

lesions in the antral portion of the stomach was used as an ulcer index on an indomethacin-produced gastric antral ulcer model. The ulcerous area (mm²) was determined under a dissecting microscope ($\times 10$) with the aid of a square grid as an ulcer index on PAF- and acetic acid-induced ulcer models.

Gastric Acid Secretion in Pylorus-Ligated Rats The rats were anesthetized with ether and the pylorus was ligated. Each sample was mixed with soybean oil and administered perorally 2 or 4 h before pylorus ligation. Four hours after the pylorus ligation, the contents of the stomach were collected and centrifuged. Compound III-1a was an oily substance and it was separated from the gastric content by centrifugation. Furthermore, compd. III-1a did not have a buffer action in itself. Then, the content was analyzed for gastric volume and total acid output. The acidity was measured by titration with 0.05 N NaOH to pH 7.0 using autotitrator (Comtite-8; Hiranuma).

Statistics Results were expressed as the mean $\pm$ S.E. and analyzed by a one-way analysis of variance. When the analysis indicated that a significance existed, the treated groups were compared to the control by Dunnett's test.

Results

Anti-ulcer Effects of Compd. III-1a on Various Experimental Ulceration Models in Rats Compound III-1a administered at an oral dose of 300 mg/kg significantly inhibited the formation of gastric ulcer induced by ethanol. On the other hand, the anti-ulcer activity of plaunotol was a reference drug was equivalent to that of compd. III-1a on ethanol-induced ulcer. Compound III-1a and plaunotol showed significant inhibitory activities in the rats having HCl-aspirin-induced gastric ulcer at 100 and 300 mg/kg, *p.o.* respectively. Both compd. III-1a and plaunotol showed a significant decrease in their indices for indomethacin-induced gastric ulcer at a dose of 100 mg/kg, *p.o.* Anti-stress ulcer activity of compd. III-1a was about 100-fold stronger

TABLE I. Effect of 2-(*E*-2-Decenoylamino)ethyl 2-(Cyclohexylethyl) Sulfide (Compd. III-1a) on Ethanol-, HCl·Aspirin-, Indomethacin- and Stress-Induced Ulceration in Rats

Ulceration	Treatment	Dose (mg/kg)	Ulcer index	Inhibition (%)
Ethanol-induced ulceration	Control ^{a,b}	—	47.5 $\pm$ 5.0	—
	Compd. III-1a ^b	300	27.8 $\pm$ 6.0 ^f	41.5
		100	35.8 $\pm$ 5.0	24.6
	Plaunotol ^b	300	28.1 $\pm$ 5.3 ^f	40.8
		100	38.0 $\pm$ 5.4	20.0
		30	45.0 $\pm$ 6.8	5.3
HCl·Aspirin-induced ulceration	Control ^{a,c}	—	44.5 $\pm$ 5.4	—
	Compd. III-1a ^c	100	22.4 $\pm$ 4.4 ^g	49.7
		30	32.1 $\pm$ 4.9	27.9
	Plaunotol ^b	300	28.9 $\pm$ 4.2 ^f	35.1
		100	29.2 $\pm$ 5.9	34.4
		30	41.9 $\pm$ 5.2	5.8
Indomethacin-induced ulceration	Control ^{a,d}	—	36.1 $\pm$ 4.7	—
	Compd. III-1a ^d	100	16.6 $\pm$ 2.6 ^g	54.0
		30	32.9 $\pm$ 4.5	8.9
	Plaunotol ^b	300	15.7 $\pm$ 2.4 ^g	56.5
		100	23.9 $\pm$ 3.4	33.8
		30	28.5 $\pm$ 3.7	21.1
Stress-induced ulceration	Control ^{a,e}	—	18.8 $\pm$ 2.1	—
	Compd. III-1a ^e	3	8.9 $\pm$ 0.9 ^g	52.7
		1	10.9 $\pm$ 1.3 ^g	42.0
	Plaunotol ^b	100	11.3 $\pm$ 1.7 ^f	39.9
		30	12.1 $\pm$ 2.0 ^f	35.6
		10	18.8 $\pm$ 2.2	0.0

All values represent the mean $\pm$ S.E. of 16 rats. a) HCO-60, 10% in saline. b) Each sample was administered perorally, 0.5 h before the peroral administration of 70% ethanol. c) Each sample was administered perorally, 0.5 h before the peroral administration of 100 mg/kg of aspirin, suspended in 1% gum arabic in 150 mM HCl. d) Each sample was administered perorally, immediately before the subcutaneous injection of 30 mg/kg of indomethacin. e) Each sample was administered perorally, immediately before the restraint and water immersion stress loading. Significantly different from the control group: f) $p < 0.05$, g) $p < 0.01$.

than that of plaunotol (Table I).

1) Effect on Indomethacin-Produced Gastric Antral Ulcer

As shown in Table II, compd. III-1a showed the same significant inhibitory activity as spizofurone did at a dose of 100 mg/kg, *p.o.* on indomethacin-produced gastric antral ulcer.

2) Effect on Hemorrhagic Shock-, DDC- and PAF-Induced Ulcer

As shown in Table III, compd. III-1a significantly inhibited the formation of gastric ulcers induced by hemorrhagic shock, DDC and PAF at doses of 100, 30 and 100 mg/kg, respectively. Plaunotol only showed significant

TABLE II. Effect of 2-(*E*-2-Decenoylamino)ethyl 2-(Cyclohexylethyl) Sulfide (Compd. III-1a) on Indomethacin-Produced Gastric Antral Ulceration in Refed Rats

Treatment	Dose (mg/kg)	Ulcer index	Inhibition (%)
Control ^a	—	3.4 $\pm$ 0.7	—
Compd. III-1a	300	1.2 $\pm$ 0.4 ^b	64.7
	100	1.6 $\pm$ 0.4 ^b	52.9
	30	2.3 $\pm$ 0.5	32.4
Spizofurone	100	1.5 $\pm$ 0.3 ^b	55.9

All values represent the mean $\pm$ S.E. of 16 rats. a) HCO-60, 10% in saline. Each sample was administered perorally 0.5 h before the refeeding. Significantly different from the control group: b) $p < 0.05$.

TABLE III. Effect of 2-(*E*-2-Decenoylamino)ethyl 2-(Cyclohexylethyl) Sulfide (Compd. III-1a) on Hemorrhagic Shock-, DDC- and PAF-Induced Ulceration in Rats

Ulceration	Treatment	Dose (mg/kg)	Ulcer index	Inhibition (%)
Hemorrhagic shock-induced ulceration	Control ^{a,b}	—	19.2 $\pm$ 2.3	—
	Compd. III-1a ^b	300	9.0 $\pm$ 1.8 ^f	53.1
		100	12.1 $\pm$ 2.5 ^e	37.0
	Plaunotol ^b	300	19.0 $\pm$ 3.7	1.0
		100	21.9 $\pm$ 3.8	(-14.1)
		30	8.1 $\pm$ 1.7	—
DDC-induced ulceration	Control ^{a,c}	—	2.6 $\pm$ 0.7 ^f	—
	Compd. III-1a ^c	100	2.9 $\pm$ 0.6 ^e	67.9
		30	5.2 $\pm$ 0.7	64.2
	Plaunotol ^c	100	7.5 $\pm$ 2.0	35.8
		30	7.5 $\pm$ 2.0	7.4
		100	6.3 $\pm$ 0.2	—
PAF-induced ulceration	Control ^{a,d}	—	4.7 $\pm$ 0.3 ^f	—
	Compd. III-1a ^d	300	5.0 $\pm$ 0.1 ^f	25.4
		100	5.0 $\pm$ 0.1 ^f	20.6
	Plaunotol ^d	300	4.8 $\pm$ 0.4 ^e	23.8
		100	5.0 $\pm$ 0.2 ^f	20.6
		30	5.0 $\pm$ 0.2 ^f	20.6

All values represent the mean $\pm$ S.E. of 8–12 rats. a) HCO-60, 10% in saline. b) Each sample was administered perorally, 0.5 h before the hemorrhagic shock. c) Each sample was administered perorally, 0.5 h before the subcutaneous injection of 1000 mg/kg of DDC. d) Each sample was administered perorally, 0.5 h before the intravenous injection of 8 μ g/kg of PAF. Significantly different from the control group: e) $p < 0.05$, f) $p < 0.01$.

TABLE IV. Effect of 2-(*E*-2-Decenoylamino)ethyl 2-(Cyclohexylethyl) Sulfide (Compd. III-1a) on Acetic Acid-Induced Ulceration in Rats

Treatment	Dose (mg/kg/d)	Ulcer index	Healing rate (%)
Control ^a	—	10.6 $\pm$ 1.2	—
Compd. III-1a	200 $\times$ 2	5.6 $\pm$ 0.7 ^c	47.2
	100 $\times$ 2	7.3 $\pm$ 0.9 ^b	31.1
	50 $\times$ 2	8.7 $\pm$ 1.1	17.9
Plaunotol	200 $\times$ 2	7.4 $\pm$ 0.8 ^b	30.2

All values represent the mean $\pm$ S.E. of 15 rats. a) Soybean oil. Each sample was administered perorally, twice a day for 14 d from the 5th day after the operation. Significantly different from the control group: b) $p < 0.05$, c) $p < 0.01$.

TABLE V. Effect of 2-(E-2-Decenoylamino)ethyl 2-(Cyclohexylethyl) Sulfide (Compd. III-1a) on Gastric Secretion in Pylorus-Ligated Rats

Treatment	Dose (mg/kg)	Gastric volume (ml/100 g b.wt.)	Total acid output ($\mu\text{eq}/100\text{ g b.wt.}$)
Control ^{a,b)}	—	2.65 $\pm$ 0.32	202.9 $\pm$ 33.1
Compd. III-1a ^{b)}	200	1.79 $\pm$ 0.23 ^{d)}	104.9 $\pm$ 23.5 ^{d)}
	100	2.63 $\pm$ 0.34	184.6 $\pm$ 33.5
	50	2.65 $\pm$ 0.32	194.0 $\pm$ 37.2
Control ^{a,c)}	—	2.34 $\pm$ 0.42	195.0 $\pm$ 44.5
Compd. III-1a ^{c)}	100	1.39 $\pm$ 0.23	88.0 $\pm$ 22.3 ^{d)}
	50	1.78 $\pm$ 0.23	124.5 $\pm$ 22.7
	25	1.58 $\pm$ 0.15	108.2 $\pm$ 16.7

All values represent the mean $\pm$ S.E. of 10 rats. a) Soybean oil. Each sample was administered perorally, 2 h^{b)} or 4 h^{c)} before pylorus ligation. Significantly different from the control group: d) $p < 0.05$. b.wt., body weight.

inhibitory activity on PAF-induced ulcer in these three ulcer models at a dose of 100 mg/kg.

3) Effect on Acetic Acid-Induced Ulcer The consecutive administration of compd. III-1a (100 mg/kg, *p.o.*) twice a day significantly accelerated the healing of acetic acid-induced ulcer and that of plaunotol (200 mg/kg, *p.o.*) showed the same activity (Table IV).

4) Effect on Gastric Acid Secretion Gastric acid secretion was significantly reduced by compd. III-1a administration at doses of 200 and 100 mg/kg, *p.o.* at 2 and 4 h before pylorus ligation, respectively (Table V).

Discussion

We reported that among the derivatives of 2-(E-2-alkenoylamino)ethyl alkyl sulfide, 2-(E-2-decenoylamino)ethyl 2-(cyclohexylethyl) sulfide (compd. III-1a) showed the strongest inhibitory activity on water immersion stress-induced ulcer and low toxicity.¹⁾ In the present study, the effects of compd. III-1a on various experimental ulcer models and gastric secretion were examined.

On ethanol-induced ulcer, ethanol directly harms gastric mucosa. Prostaglandins (PGs)¹²⁾ and leukotrienes (LTs)¹³⁾ play a significant role in the pathogenesis of this lesion. The potency of compd. III-1a on the ethanol-induced ulcer model was less than that on the other ulcer models. This result indicated that compd. III-1a did not have the cytoprotective activity by itself.

HCl·aspirin-induced ulcer is caused by the destruction of gastric mucosal barrier and the back-diffusion of H⁺.¹⁴⁾ Furthermore, it was reported that the inhibition of cyclooxygenase activity by aspirin causes the decrease of PGs in gastric mucosa and the decrease is one of the important pathogenesis of the HCl·aspirin-induced ulcer model.¹⁵⁾ From the evidence that compd. III-1a significantly inhibited this model at a dose of 30 mg/kg, it was suggested that compd. III-1a retained the gastric mucosal barrier and maintained the PGs content.

Indomethacin is one of the nonsteroidal anti-inflammatory drugs (NSAIDs) and the general side effect of its gastrointestinal injury, which is caused by the reduction of PGs content in gastric mucosa.¹⁶⁾ From the results that compd. III-1a showed the anti-ulcerous activity on ethanol-, HCl·aspirin- and indomethacin-induced ulcer models, compd. III-1a seemed to maintain or increase PGs content in gastric mucosa. Furthermore, one of the anti-ulcerous activities of compd. III-1a might be dependency on cyto-

protective activity of PGs. Further studies on PGs content and the enzyme related to PGs were scheduled.

The pathogenesis of the stress-induced ulcer model is complicated. But some of them seemed to be excitement of the autonomic nervous system,¹⁷⁾ acceleration of gastric motility,¹⁸⁾ increase of gastric secretion,¹⁹⁾ impairment of gastric mucosal blood flow,²⁰⁾ decrease of PGs²¹⁾ and reduction of cell proliferation.²²⁾ Compound III-1a showed the strongest activity on stress-induced ulcer among the gastric ulcer models we examined. This result suggested that compd. III-1a might have several effects on these pathogenesis. Further studies on them were scheduled.

Acute gastric ulcer models have the mucosal lesion in the fundic gland and the shape of the lesion is generally linear. On the other hand, the indomethacin-produced gastric antral ulcer model has the mucosal lesion in the pyloric antrum and its shape is round. From these points, the indomethacin-produced gastric antral ulcer model seems to resemble the human ulcer. It was reported that cimetidine and cetraxate did not show the effect on this model but PGE₂ did.^{23,24)} Spizofurone inhibited this ulcer model and potentiated the inhibitory effect of PGE₂ on this ulcer model.²⁴⁾ Then we used spizofurone as a reference drug on this model. From the result that compd. III-1a showed the effect on this model, compd. III-1a seemed to become the unique anti-ulcer drug.

Recently it was reported that one of the pathogenesis of the stress-induced ulcer on which there was ischemia-reperfusion in a part of gastric mucosa, was generation of oxygen free radicals.²⁵⁾ From the result that compd. III-1a showed strong inhibitory activity on the stress-induced ulcer, one of the anti-ulcerous actions of compd. III-1a seemed to be to depress the radical generation or accelerate the radical destruction. Then we examined the effects of compd. III-1a on hemorrhagic shock-, DDC- and PAF-induced ulcer models which seemed to be caused mainly by oxygen free radicals. Compound III-1a showed the significant inhibitory activities on these ulcer models and seemed to suppress the formation of the radicals or have a scavenger effect on them. But the compound which improved gastric mucosal blood flow (GMBF) was effective on these ulcer models. Then the effect of compd. III-1a on GMBF was scheduled.

There are many ulcer models but many of them are acute ulcer models. The acetic acid-induced ulcer model is thought to be the most useful chronic ulcer model for evaluating the curative effect of anti-ulcerous agents.²⁶⁾ From the result that compd. III-1a accelerated the healing rate on it, compd. III-1a seemed to have both preventive and curative effects on the ulcer.

There are two types of anti-ulcerous drugs. One type is an anti-secretory agent to which histamine H₂ receptor antagonist, cimetidine and proton pump inhibitor, omeprazole belong. The other is the so-called defensive factor promoting agents. Recently, a dual type anti-ulcer drug was investigated.²⁷⁾ Then we examined the inhibitory effect of compd. III-1a on the gastric secretion. Acid secretion in pylorus ligated rat which was thought to be basal secretion, could be inhibited significantly by the oral administration of compd. III-1a. Compound III-1a was thought to be absorbed in the duodenum or the small intestine followed by a time lag between the absorption and the action.

Furthermore, to show the inhibitory effect of compd. III-1a on the gastric secretion in pylorus ligation, compd. III-1a was thought to pass through the stomach. The administrative schedule in this time was employed from these points. The effective difference arising from the administrative schedule should be due to the absorptive rate of compd. III-1a.

The results in the present study suggest that compd. III-1a has dual action on ulcer formation.

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