EFFECT OF SYNTHETIC ACYCLIC POLYISOPRENOIDS ON THE COLD-RESTRAINT STRESS INDUCED GASTRIC ULCER IN RATS

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Abstract—The antiulcer effect of a series of synthetic acyclic polyisoprenoids on cold-restraint stress induced ulcer in female rats was determined, and the relationship between their antiulcer effect and chemical structure was also investigated. As a result, the following findings were obtained: The antiulcer effect of acyclic polyisoprenoids closely correlated with the number of intramolecular isoprene units, and geranylgeranyl derivatives showed a particularly marked antiulcer effect. The terminal polar groups such as 2-oxopropyl and 2-hydroxypropyl groups in geranylgeranylacetone seemed to play an important role in the antiulcer activity of acyclic polyisoprenoids. Terminal bulky groups decreased their antiulcer activity, however. The antiulcer activity of geranylgeranylacetone correlated with the number of intramolecular double bonds. There was no significant difference in antiulcer activity between *all*-trans-geranylgeranylacetone, 5-cis-geranylgeranylacetone and the mixture of these isomers (1:1 and 3:2). The results of this experiment suggested that the antiulcer activity of acyclic polyisoprenoids might be governed by such factors as the number of isoprene units, terminal polar groups, and number of intramolecular double bonds.

Acyclic polyisoprenoids are widely distributed in natural materials (1), but a search of the literature reveals few reports that deal with their pharmacologic effects. Adami and his co-workers (2) described the antiulcer activity of terpenoid derived from vitamin K, and Ogiso et al. (3) isolated the antiulcer terpenoid from Plau-noi, a plant used as a medicinal herb in Thailand. Spence et al. (4) reported that farnesylacetone epoxide which is chemically related to juvenile hormone isolated from marine algae had anticonvulsant action at non-sedative doses in mice. Compounds of this kind also occur in the body. Hemming (5) has demonstrated that phosphorylated acyclic polyisoprenols (e.g., polyprenylphosphate) are involved in the synthesis of glycoproteins through several sugar transfer reactions and that polyprenol occurs in biological materials as families of prenologs which differ in length from each other.

In a series of pharmacological studies on Coenzyme Q_{10} we found that this substance had an antiulcer effect on cold-restraint stress-induced and indomethacin induced gastric ulcers in rats. Later it was revealed that it was not 2,3-dimethoxy-6-methylbenzoquinon but decaprenol, an isoprenoid side chain of Coenzyme Q_{10}, that was active.

Accordingly, we assume that the isoprenoid side chain of this substance might be responsible for its antiulcer activity.

The work presented here is an investigation that was undertaken to clarify the relationship between the acyclic polyisoprenoid structure and its antiulcer effect on cold-restraint stress induced ulcer in rats.

Materials and Methods

Female rats of the Sprague-Dawley strain weighing 180 to 200 g were used in the experiment. The animals were housed in an animal breeding room maintained at 22±2°C with 55±5% relative humidity. Both food and water were restricted for 3 hr before onset of experiment. The animals were individually immobilized in each compartment of the stress cage (6), and kept in a chamber at 4°C according to the method of Brodie and Valitski (7) as slightly modified by Murakami et al. (8) After 2 hr of stress application, the animals were killed with ether, and their stomachs were removed. Following the injection of 10 ml of saline solution, the stomach was immersed in 5% neutral formalin for 5 min to fix the outer layer. An incision was made in the stomach along the greater curvature, and the lesions were examined under a dissecting microscope (×10) with a scale. The sum of individual ulcer lengths was referred to as the ulcer index.

The acyclic polyisoprenoids (*all*-trans type) used in this investigation were synthesized by the Eisai Co. In the comparative study of geranylgeranylacetone-isomers at the 5-position, 5-cis, 9-trans, 13-trans-geranylgeranylacetone was also synthesized. Gefarnate was purified from Gefanil® and retinol obtained from the Wako Co. The test article was emulsified with 5% gum arabic and 0.6% Tween 80 and administered by gavage 30 min before stress application. The dosage level of 200 mg/kg which was previously shown to be effective in the studies with Coenzyme Q_{10} was used in this investigation.

The data were assessed for the significance of difference by the Student's *t*-test.

Results

After cold-restraint stress was applied to the animals for 2 hr many superficial erosions of varying length occurred in the gastric fundic mucosa. The mean ulcer index obtained in the control animals was 11.2±0.7. The gross findings of ulcers induced by cold-restraint stress in rats are shown in Fig. 1.

1. Number of isoprene units in acyclic isoprenoid compounds and their antiulcer activity: The antiulcer activity was compared among three acyclic polyisoprenoid homologs polyprenylacetones, ethylpolyprenoates, and polyprenols, at a dosage level of 200 mg/kg. The data were expressed as rates of inhibiton as compared to the ulcer indexes obtained in the control animals.

The antiulcer activities of polyprenylacetones of differing isoprene unit number (n) are shown in Fig. 2A. Compound 1 (n=3, *all*-trans-farnesylacetone, C-1) and compound 2 (n=4, *all*-trans-geranylgeranylacetone, C-2) showed a marked antiulcer effect (rates of ulcer inhibition, 68 and 72%, respectively). When the number of isoprene

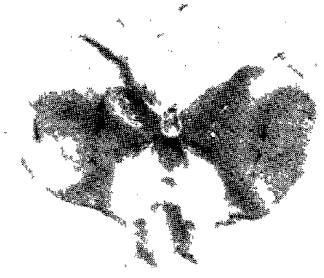


Fig. 1. Gross appearance of stress-ulcers induced by cold-restraint (at 4°C, for 2 hr) in a rat. Gastric erosions are localized at the glandular portion, and the shape of erosion is almost linear.

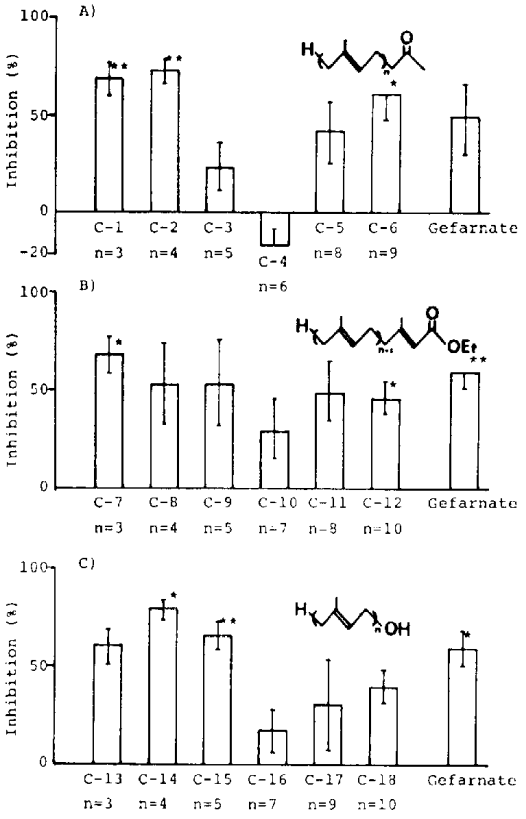


Fig. 2. Relationships between the number of isoprene units (n) in acyclic polyprenylacetone (A), acyclic ethylpolyprenoates (B), and acyclic polyprenylalcohols (C) and the antiulcer activity on cold-restraint stress ulcer in rats. Test compounds were administered orally at a dose of 200 mg/kg, 30 min before the exposure to the stress. Each data value represents the inhibitory rate (mean±S.E.) to the corresponding control index (*$P<0.05$ and **$P<0.001$). Ten animals were used in each group.

units was increased to 5 (compound 3, *all*-trans-farnesylgeranylacetone, C-3) or 6 (compound 4, *all*-trans-farnesylfarnesylacetone, C-4), the antiulcer activity of the compounds was decreased. An antiulcer effect was also noted in compound 5 (n=8, *all*-trans-farnesylfarnesylgeranylacetone, C-5) or compound 6 (n=9, *all*-trans-farnesylfarnesylfarnesylacetone, C-6) (rates of inhibition, 41 and 60%, respectively). Similar results were obtained with ethylpolyprenoates and polyprenols (Fig. 2B and 2C).

2. Antiulcer activity of geranylgeranyl derivatives: Geranylgeranyl derivatives were obtained by substituting the terminal groups of acyclic polyisoprenoids, and their antiulcer effect was compared at a dosage level of 200 mg/kg, as shown in Table 1.

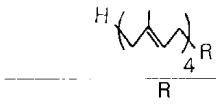
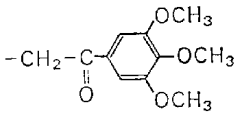
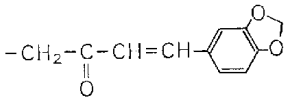
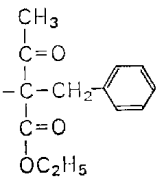
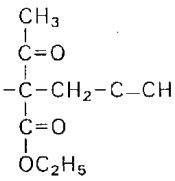
As noted in the preceding experiment, compound C-2 showed a marked antiulcer effect (rate of inhibition, 70.7%). When the 2-oxopropyl group of compound C-2 was substituted with the 2-oxopentyl group (C-19) or the 3-methyl-2-oxybutyl group (C-20), its antiulcer activity was reduced to some extent. When the terminal group of compound C-2 was replaced with a bulkier one as in compounds C-21, C-22, C-23 and C-24, the antiulcer activities of the compounds were reduced by a remarkable degree. When the terminal group of C-2 was substituted with an amino group (C-25) or a carbethoxyl amino group (C-26), the compound retained antiulcer activity, though it was weak. Compound C-27 that has no polar group only showed slight antiulcer activity. Compound C-28 having the acetoxy group no longer had antiulcer activity. The alcohol compound C-29 derived by hydrogenating the ketone in compound C-2, showed about the same antiulcer activity as compound C-2. Retinol showed remarkable antiulcer activity.

3. Antiulcer activity of geranylgeranyl derivatives and its relationship to the number of intramolecular carbon-carbon double bonds: The antiulcer activity was compared among geranylgeranyl derivatives with a differing number of intramolecular double bonds at dosage levels of 50 to 200 mg/kg (Table 2).

Compound C-30 having the conjugated carbonyl group showed the most marked antiulcer activity. Even at the lowest dosage level of 50 mg/kg its rate of ulcer inhibition was as significant as 60%. On the other hand, compound C-32 that has only one intra-

molecular double bond at the 5 position showed a weak antiulcer activity. Even at the highest dosage level of 200 mg/kg, its rate of ulcer inhibition was 60%. Compound C-33 in which all the double bonds are saturated showed a still lower antiulcer

Table 1. Effect of geranylgeranyl derivatives on cold restraint stress ulcer in female rats

		Dose mg/kg	Number of rats	Inhibition (%)
C-2	$-CH_2-C(=O)-CH_3$	200	10	70.7±5.2**
C-19	$-CH_2-C(=O)-CH_2-CH_2-CH_3$	200	10	53.4±9.6*
C-20	$-CH_2-C(=O)-CH<^{CH_3}_{CH_3}$	200	10	56.1±9.4*
C-21		200	10	−3.1±15.5
C-22		200	10	8.2±22.7
C-23		200	10	38.8±5.8
C-24		200	10	23.0±14.4
C-25	$-NH_2$	200	10	53.0±16.7
C-26	$-NH-C(=O)-OC_2H_5$	200	10	43.9±21.2
C-27	$-H$	200	10	23.5±18.2
C-28	$-O-C(=O)-CH_3$	200	10	12.8±14.0
C-29	$-CH_2-CH(OH)-CH_3$	200	10	86.5±5.7**
Retinol acetate		200	10	78.0±7.5**

*$P<0.05$ and **$P<0.01$ when compared with the control. Each value represents the mean±S.E.

Table 2. Effect of related compounds of geranylgeranylacetone with different number of double bonds on cold restraint stress ulcer in rats

	Structure	Number of double bonds	Number of animals	Dose mg/kg	Inhibition (%)
C-30		5	10	50	61 8± 7.7**
			10	100	83.2± 3.2***
			10	200	90.5± 3.2***
C-2		4	10	50	56.9± 9.9*
			10	100	79.6± 5.3***
			10	200	71.8± 6.0**
C-31		2	10	50	37.7±16.7
			10	100	61.5± 7.7
			10	200	83.8± 5.5**
C-32		1	10	50	32.0±19.2
			10	100	60.0± 8.8**
			10	200	60.8±12.8*
C-33		0	10	50	4.7±23.6
			10	100	52.0±11.0
			10	200	52.6± 9.3*

*P<0.05, **P<0.01, ***P<0.001. Each value represents the mean±S.E.

Table 3. Effect of isomers of geranylgeranylacetone on cold-restraint stress induced gastric ulcer in female rats

Treatment	Dose mg/kg	N	Ulcer Index Mean±S.E.	Inhibition %
Control	—	10	9.9±1.8	—
A	50	10	3.6±0.8**	63.6
	100	10	2.7±0.8**	72.7
B	50	10	4.1±1.1*	58.6
	100	10	1.3±0.4**	86.9
C	50	10	2.9±0.9**	70.7
	100	10	1.5±0.5**	84.8
Control	—	20	8.8±0.9	—
D	50	10	3.1±0.5**	64.8
	100	10	2.3±0.9**	73.9

A: *all*-trans-geranylgeranylacetone. B: 5-cis-geranylgeranylacetone (cis 95%). C: A-B mixture (A:B=1:1). D: A-B mixture (A:B=3:2). *P<0.05 and **P<0.01 when compared with the control.

activity.

Antiulcer activity of these compounds was in the order of C-29 (number of carbon-carbon bonds=5) > C-2(n=4) > C–30 (n=2) > C-31(n=1) > C-32(n=0). The antiulcer activity of these compounds showed close coincidence with the number of intramolecular double bonds.

4. Antiulcer activity of trans- and cis-isomers of geranylgeranylacetone: Although eight geometrical isomers are possible for geranylgeranylacetone, the compounds used in this study differ in stereochemistry only at the 5-position, the other double bond stereo-

chemistry being fixed trans. Thus, the antiulcer activity was compared between trans-isomer (A), i.e., *all*-trans-geranylgeranylacetone, and cis-isomer (B) that differ in stereochemical configuration only at the 5-position (Table 3).

Isomer A inhibited the cold-restraint stress-induced ulcer in 63.6 and 72.7% of animals at dosage levels of 50 and 100 mg/kg, and isomer B, 58.6 and 86.9%, there being no significant difference in rate of inhibition between A and B. The 1:1 mixture of isomers A and B inhibited the ulcer in 70.7 and 84.8% of animals at dosage levels of 50 and 100 mg/kg. The 3:2 mixture of isomers A and B which is the product of reaction in the geranylgeranylacetone synthesis from *all*-trans-geranyllinalool, inhibited the ulcer at the same rate as A alone did. Again there was no significant difference in ulcer inhibition, no matter whether isomers A and B were used alone or combined together.

Discussion

The antiulcer activity of acyclic polyisoprenoids with a differing number of isoprene units was studied with the cold-restraint stress induced ulcer model. As a result, it was revealed that three types of acyclic polyisoprenoids, polyprenylacetones, ethylpolyprenoates, and polyprenylalcohols, that have 3 to 4 or 8 to 10 isoprene units exhibited antiulcer activity. Among others, *all*-trans-geranylgeranylacetone (C-2) and *all*-transgeranylgeraniol (C-14) were found to have particularly marked antiulcer activity, and the 1:1 and 3:2 mixtures of *all*-trans-geranylgeranylacetone and its 5-cis-isomers showed the same antiulcer activity as *all*-trans-geranylgeranylacetone did. These results are of great interest in the light of our previous report (9) that geranylgeranylacetone derivatives promote the biosynthesis of glycolipid intermediates that are thought to play a role in the formation of glycoproteins and the report of Mańkowski et al. (10) that phosphorylated solanesol (with 9 isoprene units) is involved in the synthesis of glycoproteins. On the other hand, farnesylgeranyl and farnesylfarnesyl compounds having 5 or 6 isoprene units showed as weak antiulcer activity as the three kinds of polyisoprenoids. This fact indicates that the number of isoprene units is an important factor for the antiulcer activity of acyclic polyisoprenoids.

Of these compounds, *all*-trans-geranylgeranylacetone (C-2) showed the most remarkable antiulcer activity, but when its 2-oxopropyl group was substituted with the 2-oxopentyl group, 3-methyl-2-oxobutyl group, amino group, carbetoxyamino group or some other bulkier group, its antiulcer activity was reduced to a remarkable extent. Of the geranylgeranyl derivatives, on the other hand, *all*-trans-geranylgeraniol and *all*-trans-ethylgeranylgeranoate showed about the same antiulcer activity as *all*-trans-geranylgeranylacetone. This finding suggests that relatively polar groups such as carbonyl and hydroxyl groups are important to the antiulcer activity of geranylgeranyl derivatives, and this assumption is in agreement with the postulation of Adami et al. (2) who maintain that the presence of a polar group in the terpenic molecule is important.

The study of the relationship between the antiulcer activity of *all*-trans-geranylgeranylacetone (C–2) and its number of intramolecular double bonds revealed that C–30 with 5 intramolecular double bonds had a higher degree of antiulcer activity than any other geranyl-geranylacetone related compounds and that perhydrogeranylgeranylacetone (C–33) with saturated bonds had the weakest antiulcer activity. The sequence of increasing antiulcer activity of these compounds was in agreement with the order of increasing number of intramolecular double bonds. Adami et al. (2) reported that tetrahydrogeranylfarnesylacetate and hexahydrofarnesylfarnesylacetate had lower antiulcer

activity than their corresponding unsaturated compounds (geranylfarnesylacetate and farnesylfarnesylacetate). Their finding suggests that the number of intramolecular carbon-carbon double bonds is associated with the antiulcer activity of these compounds. The relationship between the position of intramolecular double bonds and the antiulcer activity of compounds has not been clarified sufficiently, and further studies should be conducted to obtain more extensive data.

The results of this experiment indicate that the chain length plays an important role in the pharmacological action of acyclic polyisoprenoids. It therefore seems possible that they exhibit a specific action in the body.

It is known that polyisoprenoids (11) like dolichol (with 17 to 21 isoprene units) that is saturated at the α-position and has a relatively long chain length occurs as prenolog in the body and that its compounds play a role as intermediates used in the process of glycoprotein synthesis (5). Recently it was reported that retinol, one of geranylgeranyl derivatives, that has a shorter chain length than dolichol was involved in the process of glycoprotein synthesis as an intermediate (12). De Luca and Wolf (13) suggested that vitamin A functioned in the small intestinal cell membrane as a sugar carrier lipid intermediate for the biosynthesis of glycoproteins. On the other hand, Schumpelick and Farthmann (14) reported that retinol or carotinoids inhibited restraint stress induced ulcer in rats. However, the mechanism by which retinol exhibits this antiulcer effect has not been clarified sufficiently. Nor has been elucidated fully the relationship between gastric ulcer formation and biosynthesis of glycoproteins. It has been assumed for long time, however, that mucus composed of glycoproteins protects the gastric mucosa against the ulcer forming factors. As a matter of fact, we previously reported that a reduction in levels of glycoproteins and hexosamine in the mucous layer of the stomach was associated with the development of cold-restraint stress ulcer and that geranylgeranylacetone prevented the reduction in hexosamine level in the mucous layer of the stomach (15, 16). Sander et al. (17) postulated that a remarkable reduction in hexosamine synthetase activity in the gastric mucosa might be associated with the development of gastric ulcer. These observations seem to provide suggestive evidence that acyclic polyisoprenoids play an important role in strengthening the gastric defense mechanism against gastric mucosal injury.

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References

1) **Clayton, R.B.:** The biological significance of the terpenoid pathway of biosynthesis. *In* Aspects of Terpenoid Chemistry and Biochemistry, Edited by Goodwin, T.W., p. 1–23, Academic Press, London and New York (1971)
2) **Adami, E., Marrazzi-Uberti, E. and Turba, C.:** The anti-ulcer action of some natural and synthetic terpenic compounds. Med. Exp. **7**, 171–176 (1962)
3) **Ogiso, A., Kitazawa, E., Kurabayashi, M., Sato, A., Takahashi, S., Noguchi, H., Kuwano, H., Kobayashi, S. and Mishima, H.:** Isolation and structure of antipeptic ulcer diterpene from Thai medicinal plant. Chem. Pharm. Bull. (Tokyo) **26**, 3117–3123 (1978)
4) **Spence, I., Jamieson, D.D. and Taylor, K.M.:** Anticonvulsant activity of farnesylacetone epoxide—a novel marine natural product. Experimentia **35**, 238–239 (1979)
5) **Hemming, F.W.:** Lipids in glycan biosynthesis. *In* MTP International Reviews in Science, Biochemistry, Series 1: Biochemistry of Lipids, Edited by Goodwin, T.W., Vol. **4**, p. 39–98, Butterworths, London (1974)
6) **Takagi, K. and Okabe, S.:** The effects of drugs on the production and recovery processes of the stress ulcer. Japan. J. Pharmacol. **18**, 9–18 (1968)
7) **Brodie, D.A. and Valitski, L.:** Production of gastric hemorrhage in rats by multiple stresses. Proc. Soc. Exp. Biol. Med. **113**, 998–1001 (1963)

8) **Murakami, M., Oketani, K., Fujisaki, H., Wakabayashi, T., Ohgo, T. and Kawai, K.:** Fundamental studies of the changes of gastric mucosubstances induced by cold-restraint stress in rats. J. Kyoto Pref. Univ. Med. **90**, 443–451 (1981)
9) **Murakami, M., Muto, Y., Moriwaki, M. and Ohgo, T.:** Effect of acyclic polyisoprenoids on the biosynthesis of mannose-labeled glycolipids in rat liver microsomes. Biochem. Biophys. Res. Commun. **103**, 706–712 (1981)
10) **Mańkowski, T., Sasak, W. and Chojnacki, T.:** Hydrogenated polyprenol phosphates—exogenous lipid acceptors of glucose from UDP-glucose in rat liver microsomes. Biochem. Biophys. Res. Commun. **65**, 1292–1297 (1975)
11) **Richards, J.B. and Hemming, F.W.:** The transfer of mannose from guanosine diphosphate mannose to dolichol phosphate and protein by pig liver endoplasmic reticulum. Biochem. J. **130**, 77–93 (1972)
12) **Rosso, G.C., De Luca, L.M., Warren, C.D. and Wolf, G.:** Enzymatic synthesis of mannosyl retinyl phosphate from retinyl phosphate and guanosine diphosphate mannose. J. Lipid Res. **16**, 235–243 (1975)
13) **De Luca, L.M. and Wolf, G.:** Vitamin A and mucus secretion: a brief review of the effect of vitamin A on the biosynthesis of glycoproteins, Int. Vit. Res. **40**, 284–290 (1970)
14) **Schumpelick, V. and Farthmann, E.:** Untersuchung zur protektiven Wirkung von Vitamin A bein Stressulkus der Ratte. Arzneimittelforsch. **26**, 386–388 (1976)
15) **Murakami, M., Oketani, K., Fujisaki, H., Wakabayashi, T., Ohgo, T. and Kawai, K.:** Fundamental studies of the changes of gastric mucosubstances induced by cold-restraint stress in rats. J. Kyoto Pref. Univ. Med. **90**, 443–451 (1981)
16) **Murakami, M., Oketani, K., Fujisaki, H., Wakabayashi, T., Ohgo, T. and Kawai, K.:** Effects of geranylgeranylacetone on the changes of gastric mucosubstances in rats subjected to cold-restraint stress. J. Kyoto Pref. Univ. Med. **90**, 453–459 (1981)
17) **Sander, L.D., Chandler, A.M. and Johnson, L.R.:** Changes in liver and gastric mucosal hexosamine synthesis after restraint. Gastroenterology **68**, 285–293 (1975)