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Anti-inflammatory Activities of Pyridylalanine Analogues and Their Influences on the Anti-inflammatory Action of Cortisone

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1) Anti-inflammatory activities of pyridylalanine analogues were more potent than phenylbutazone (PB) in the carrageenin-induced rat paw edema test. *dl*-3-Pyridylalanine (3-PA) was 3 times more active than *dl*-2-pyridylalanine (2-PA) and 4 times more active than *dl*-4-pyridylalanine (4-PA) in this test.

2) The effects of PA analogues on the development of granuloma were studied in adrenalectomized rats by the cotton pellet method. 2-PA reduced the development of granuloma when given singly to rats. A simultaneous administration of 2-PA with cortisone clearly enhanced the anti-granulomatous action of cortisone. A single administration of 3-PA did not affect the development of granuloma, while a simultaneous administration of 3-PA with cortisone inhibited the action of cortisone. 4-PA had no effect on the action of cortisone and it did not affect the granuloma formation when administered alone.

3) The effects of PA analogues on the exudation were examined by the granuloma pouch method, using non-adrenalectomized rats. Among the PA analogues, only 2-PA showed a significant anti-exudative action when given singly to rats. The action of 2-PA was more active than that of PB, but less active than cortisone. The action of cortisone was enhanced by 2-PA, however, hardly affected by simultaneous administration of 3-PA or 4-PA.

Keywords——pyridylalanine; glycyrrhizin; phenylbutazone; cortisone acetate; anti-inflammatory activity; influence on cortisone action; cotton pellet granuloma; exudate in granuloma pouch; carrageenin-induced edema

Phenylglycine *n*-heptyl ester has been shown in both *in vitro* and *in vivo* tests to be a potent inhibitor of bradykinin, 5-hydroxytryptamine (5-HT), histamine and dextran in rats and of aceytlcholine, histamine, 5-HT and anaphylaxis in guinea pigs.[2)] More recently, this compound and the corresponding ester of phenylalanine were found to inhibit carrageenin and dextran responses in rat paws as well as arthritis induced by Freund's adjuvant in rats.[3)] The knowledge that the known α-amino acids containing heterocyclic ring system are intimately associated with a number of important biological processes has led us to consider the chemical and pharmacological properties of α-amino acids containing pyridine ring, which has not been found in any naturally occurring α-amino acids but is present in other compounds isolated from natural sources.

Kumagai, *et al.* reported that glycyrrhizin (GL), which is one of effective chemical constituents of licolice root, inhibited some biological actions of cortisone, *e.g.* liver glycogen deposition and anti-granulomatous action.[4)] In a previous report,[5)] we presented that *dl*-3-pyridylalanine (3-PA) inhibited the deposition of liver glycogen by glucocorticoid similar to the effect of GL.

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The present investigation was carried out to study how PA analogues influenced on some inflammations when given singly or simultaneously with cortisone to rats, comparing with phenylbutazone and GL.

Materials and Methods

Materials

dl-2-Pyridylalanine (2-PA), mp 204—206°, *dl*-3-pyridylalanine (3-PA), mp 261—262°, and *dl*-4-pyridylalanine (4-PA), mp 235—236°, were synthesized by the method of Niemann, *et al.*[6] These compounds were dissolved in 0.9% NaCl and used for injection. Cortisone acetate (Upjohn Co.), Glycyrrhizin (Minophagen Co.), phenylbutazone (Ciba-Geigy Co.), croton oil (Wako Junyaku Co.) and carrageenin (Pasco Co.) were obtained from commercial source.

Methods

Carrageenin-induced Edema——Anti-edema activity was determined by the method of Winter, *et al.*[7] A 0.5% solution of carrageenin in 0.9% NaCl was prepared at 16—20 hr before use. The test compounds were injected intraperitoneally 30 min. prior to the subplantar injection of 0.1 ml of 0.5% carrageenin into the left hind paws of male rats (Wistar, 135—165 g). The paw volume was measured at 0, 1.5, 3 and 5 hr after the carrageenin injection, using a plethysmometer (Model ΔV3, Ugo Basile, Milano, Italy).

Cotton Pellet Granuloma——Anti-granulomatous action was studied by use of cotton pellet method which was originated by Meier, *et al.*[8] and modified by Nanaboshi.[9] Cotton pellets (weight range 50±1 mg) prepared from unbleached cotton were bilaterally implanted subcutaneously into the upper dorsal area following adrenalectomy. The test compounds were injected subcutaneously into the hips of female rats (Wistar, 140—170 g). After 7 days, the rats were decapitated, and the pellets were removed from surrounding tissues and weighed after being dried overnight at 65°.

Granuloma Pouch Method——Anti-exudative action was measured by the method of Robert and Nezamis[10] using female rats (Wistar, 135—165 g). Daily injections of the test compounds were started on the day of granuloma pouch formation and the volume of exudate in the pouch was measured on the 7th day.

Table I. Effects of Pyridylalanine (PA) Analogues, Phenylbutazone (PB) and Glycyrrhizin(GL) on Carrageenin-induced Edema, 6 Animals per Group

Compound	Dose (mg/kg)	% inhibition		ID_{50}[b] (mg/kg)
		3 hr[a]	5 hr[a]	
2-PA	50	32.0	27.5	150
	100	46.5	37.5	
	250	56.0	42.1	
3-PA	50	36.9	48.4	58
	100	37.9	57.3	
	250	45.3	68.1	
4-PA	50	26.5	17.4	231
	100	37.9	29.2	
	250	51.6	38.5	
PB	50	16.8	13.5	—
	100	31.0	28.1	
	250	Death	Death	
GL	50	0	1.5	—
	100	6.7	18.0	
	250	18.8	20.8	

a) Time after carrageenin injection.
b) Intraperitoneal ID_{50} value of 3-PA was calculated by the use of value at 5 hr and the other analogues by the use of values at 3 hr.

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Results

Effects of Pyridylalanine (PA) Analogues, Phenylbutazone (PB) and Glycyrrhizin (GL) on Carrageenin-induced Edema

The swelling of the carrageenin-induced paws in control rats reached to maximum at 3 hr after the injection and continued for another 2 hr. PA analogues produced dose-dependent inhibition of edema which were maintained throughout the duration of the experiments as shown in Table I. The anti-edematous action of PA analogues appeared to be more active than that of PB. 3-PA showed the most potent inhibition of edema at 5 hr after the carrageenin treatment. In case of 2-PA, 4-PA and PB, the maximum of anti-edematous action was observed at 3 hr after the carrageenin injection. GL had no significant inhibitory effect on this experimental edema.

Table II. Effects of Pyridylalanine (PA) Analogues, Phenylbutazone (PB), Glycyrrhizin (GL) and Cortisone Acetate on Granuloma Formation, Alone and in Combinations, 10 Pellet per Group

Compound	Dose mg/100g/day	% increase of granuloma weight (Mean ±S.E.)	Student "t" test
Control (Saline)		236±5.8	
Cortisone	1.25	174±4.5	$p<0.001^{a)}$
2-PA	5.0	187±5.3	$p<0.001^{a)}$, N.S.$^{b)}$
3-PA	5.0	234±7.8	N.S.$^{a)}$
4-PA	5.0	242±6.2	N.S.$^{a)}$
PB	5.0	191±5.3	$p<0.001^{a)}$
GL	5.0	233±7.7	N.S.$^{a)}$
Cortisone +2-PA	1.25 10.0	145±5.2	$p<0.001^{b)}$
Cortisone +3-PA	1.25 10.0	229±9.2	N.S.$^{a)}$, $p<0.001^{b)}$
Cortisone +4-PA	1.25 10.0	168±6.8	N.S.$^{b)}$
Cortisone +PB	1.25 10.0	148±3.7	$p<0.001^{b)}$
Cortisone +GL	1.25 10.0	227±5.3	N.S.$^{a)}$, $p<0.001^{b)}$

a) Compared with control group.
b) Compared with cortisone group.

Effects of PA Analogues, PB, GL and Cortisone Acetate on Granuloma Formation

The results were shown in Table II. Cortisone clearly reduced the dry weight of granuloma at a dosage of 1.25 mg. 2-PA (5.0 mg) and PB (5.0 mg) also inhibited the development of granuloma and their inhibitory effects were considerable as the effect of cortisone (1.25 mg). A single administration of 3-PA or 4-PA hardly influenced the development of granuloma. GL did not also affected the granuloma formation when given singly to rats.

Simultaneous administration of 2-PA or PB with cortisone significantly enhanced the anti-granulomatous action of cortisone, while 3-PA inhibited the action of cortisone. The action of cortisone was also blocked by GL in a dosage 8 times that of cortisone. 4-PA hardly influenced the action of cortisone at the dosage studied.

As shown in Fig. 1, the inhibitory effect of 3-PA was nearly maximum at a dosage twice that of cortisone. A complete inhibition was observed at 4 times dosage.

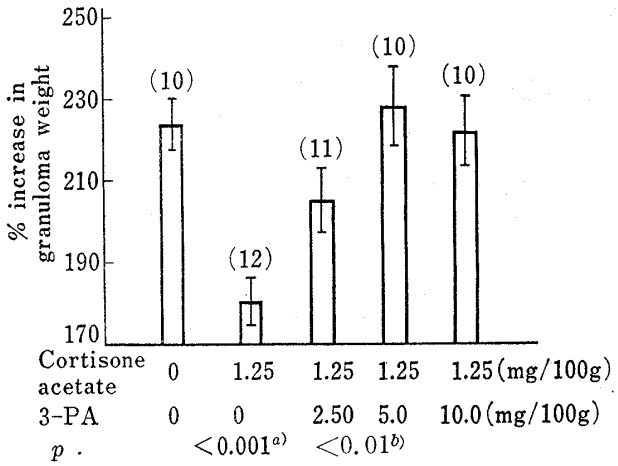

Fig. 1. Inhibitory Effect of 3-Pyridylalanine (3-PA) on the Anti-granulomatous Action of Cortisone

Numbers in parentheses indicate the numbers of pellets.
Vertical bars represent the mean ±S.E.
a) Compared with control group.
b) Compared with cortisone group.

Effects of PA Analogues, PB, GL and Cortisone Acetate on Exudation

The results shown in Table III indicate that a single administration of cortisone and 2-PA had an inhibitory effect on the exudation. The anti-exudative activity of 2-PA (10.0 mg) was considerable as the activity of cortisone (1.25 mg). The other PA analogues, 3-PA and 4-PA had no significant effect on the volume of exudate. A single administration of PB and GL also had no effect on the exudation.

Simultaneous administration of 2-PA with cortisone gave more potent anti-exudative effect than a single administration of 2-PA and cortisone. It was also found that the action of cortisone was hardly affected by 3-PA and 4-PA. The action of cortisone slightly enhanced by PB, but the effect of PB was not significant. Simultaneous administration of GL did not inhibit the effect of cortisone in inhibiting the exudation.

Table III. Effects of Pyridylalanine (PA) Analogues, Phenylbutazone (PB), Glycyrrhizin (GL) and Cortisone Acetate on the Volume of Exudate in the Granuloma Pouch, Alone and in Combinations

Compound	Dose mg/100g/day	No. of animals	ml of the exudate (Mean ±S.E.)	Student "t" test
Control (Saline)		5	7.52±0.66	
Cortisone	1.25	5	3.12±0.33	$p<0.001^{a)}$
2-PA	5.0	5	5.71±0.63	N.S.$^{a)}$
	10.0	5	3.96±0.49	$p<0.01^{a)}$, N.S.$^{b)}$
3-PA	10.0	5	7.33±0.46	N.S.$^{a)}$
4-PA	10.0	5	6.23±0.51	N.S.$^{a)}$
PB	10.0	4	5.18±0.99	N.S.$^{a)}$
GL	10.0	5	8.83±0.61	N.S.$^{a)}$
Cortisone +2-PA	1.25 10.0	4	0.65±0.65	$p<0.01^{b)}$
Cortisone +3-PA	1.25 10.0	5	2.51±0.38	N.S.$^{b)}$
Cortisone +4-PA	1.25 10.0	5	1.93±0.44	N.S.$^{b)}$
Cortisone +PB	1.25 10.0	5	2.16±0.59	N.S.$^{b)}$
Cortisone +GL	1.25 10.0	4	3.88±0.78	N.S.$^{b)}$

a) Compared with control group.
b) Compared with cortisone group.

Discussion

Thomas, *et al.*[3] reported that phenylglycine and phenylalanine *n*-heptyl esters inhibited carrageenin and dextran responses in rat paws, and both the amino acids and the *n*-heptanol

were inactive. Bailey, *et al.*[11] showed that cysteine and certain other thiol-containing compounds are effective anti-inflammatory agents. However, the anti-inflammatory activities of free α-amino acids except cysteine are not yet found.

In the present experiment, it was observed that PA analogues (heterocyclic amino acids) showed more potent anti-inflammatory activities than PB on the carrageenin-induced edema.

When simultaneously administered with cortisone to rats, PA analogues had various effects on the anti-granulomatous and anti-exudative actions of cortisone. Simultaneous administration of 2-PA and cortisone gave more potent anti-granulomatous and anti-exudative actions than a single administration of 2-PA or cortisone. 4-PA had no effect on the action of cortisone. 3-PA had an inhibitory effect on the anti-granulomatous action of cortisone without any influence on the anti-exudative action of cortisone. Although GL and 3-PA are unlike in their chemical structure, the effect of 3-PA on the action of cortisone was similar to that of GL.

Kumagai, *et al.* reported that GL inhibited the following biological actions of cortisone[4,12]: anti-granulomatous action, supression of ACTH biosynthesis in the pituitary, liver glycogen deposition, induction of liver tryptophan pyrrolase and thymolitic action. They suggested that glycyrrhetinic acid, which was an aglycone of GL and had a similar structure to steroid hormone, exerted its inhibitory effect through competition with cortisone.[4a]

Considering the zwitterionic structures, one would expect that in all of PA analogues the positively charged ammonium nitrogen atom would try to get as close as is structurally possible to the negatively charged pyridine nitrogen atom. In the case of 2-PA this tendency would lead to the formation of a strong intramolecular hydrogen bond between the ammonium nitrogen atom and the pyridine nitrogen atom. Therefore, Niemann, *et al.*[6] described that pyridine nucleus, in all three PA analogues, was less basic than pyridine, and that the basicity of the pyridine nucleus increased as the amino acid side chain was shifted progressively from the 2 to the 4 position with the greatest increment accompanying the shift from the 2 to the 3 position. Furthermore, Niemann, *et al.* reported that the increase in acid strength of carboxy group as well as the decrease in basic strength of amino group can be explained in terms of the inductive effect of the pyridine nucleus.

These differences of chemical properties of PA analogues seem to be a fact to explain the action mechanisms of these analogues which showed the different responces on the action of cortisone. However, the elucidation of action mechanism of PA analogues is the subject for a later study with the other effects of these analogues and their derivatives on the inflammation and the action of glucocorticoids.

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