# ANTI-INFLAMMATORY AND ANALGESIC PROPERTIES OF FOUR AMINO-ACIDS

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**Summary** : Orally administered L–isoleucine, DL-isoleucine and L–leucine exhibited anti-inflammatory activity in many test models of inflammation except formaldehyde-induced inflammation. L-β-phenylalanine inhibited carrageenan-induced oedema only. L-isoleucine also exhibited prolonged analgesic effect while DL-isoleucine had a short lasting effect. The amino-acids produced no gastric ulceration or overt acute toxicity in doses which effectively suppress inflammation. Anti-inflammatory activity seems to be related with interferance with the action and/or synthesis of prostaglandins and deserves further intensive study.

**Key words** : amino-acids, carrageenan-oedema, formaldehyde-oedema, 5HT-oedema, nystatin-oedema, granuloma pouch

## INTRODUCTION

Recent observations suggest that a number of amino-acids such as L–tryptophan (4), L–phenylalanine (6), cysteine (26), creatine (12), DL-valine (13), DL-tryptophan (18), creatinine (19), aspartic acid, alanine and glycine (21), L-glutamine (11), L-methionine (14), and L-valine (15), possess anti-inflammatory activity against experimentally induced inflammation. The present report describes the anti-inflammatory and analgesic properties of 4 amino-acids, so far unexplored.

## MATERIAL AND METHODS

*Drug administration in rats :* Albino rats (Haffkine strain) of either sex (90-150 g) were used in groups of 6-10 rats each.

The amino-acids (L-isoleucine, DL-isoleucine, L-leucine and DL-β-phenylalanine : SD's Lab, Chem. Industry, Bombay) and the standard reference drug, phenylbutazone (Suhrid Geigy Ltd, Bombay). were administered orally as aqueous suspension in 2% gum acacia solution in volume not exceeding 2.5 *ml/100 g*. Control group received 0.5 *ml/100 g* of vehicle alone, Student's 't' test was used for statistical evaluaion. $ED_{50}$ values were calculated by the method of Miller and Tainter (20).

**Inflammation models**

*Pedal oedema* : Rats given either the vehicle or a drug 30 min before were injected with 0.1 *ml* of a phlogistic agent (in distilled water) under plantar aponeurosis of the right paw. The paw volumes were measured immediately after the injection and again at various intervals (as specified below) with a plethysmometer, and difference considered as oedema volume. The phlogistic agents and the time of oedema assessment were as below :

(a) a 1% solution of calcium carrageenan (3 hr) ; (b) a 0.5% solution of 5-hydroxytryptamine (5-HT) creatinine sulphate (1½ and 4 hr) ; (c) formaldehyde (2%, v/v) (1½, 24 and 48 hr) and (d) a 6% suspension of nystatin in distilled water (2, 4, 6, 24, 48 and 72 hr).

*Granuloma pouch* : This was induced as described by Madan *et al.* (17). 20 *ml* of air, followed by 0.5 *ml* 2% croton oil in ground nut oil, were injected into the loose connective tissue between the shoulder blades of rats. Drug treatment was given daily for 7 days begining one day prior. On the 8th day the animals were anaesthetized with ether and the pouch was opened. The volume of the exudate in the pouch was measured and the dissected pouch weighed. The weights of the adrenals were also determined and the stomach was examined for the presence of ulcers.

**Castor oil diarrhoea**

The test was performed as described by Niemegeers *et al.* (22) in albino rats. They were given the vehicle or a test substance p o 1 hr before a dose of castor oil (1 *ml*/ *100 g*). Faeces were collected on white papers from individual animals and number of droppings at 3 hr were used in comparisons.

**Analgesic activity**

Drugs were also tested for analgesic activity in rats employing Techno Analgesiometer (5). The reaction time (tail withdrawl) was recorded before and 30 min, 1, 2 and 3 hr after administration of the drugs.

**Toxicity studies**

Male rats (100-200 g) were given an amino-acid (0.5 *g/kg*, 1 *g/kg* or 2 *g/kg* p o) and were closely observed for 4 hr for signs of overt toxicity (10) and for overnight mortality. After four weeks all animals were sacrificed and complete autopsy was performed.

## RESULTS

**Pedal oedema tests**

Carrageenan oedema was significantly reduced by all amino-acids in dose related manner. $ED_{50}$, thus found (Table I ), were employed in further work as a single dose.

5-HT-oedema was inhibited by all drugs except DL-β-phenylalnine (Table II). Formaldehyde-oedema was maximal at 48 hr (oedema volume, 0.71 ± 0.13 *ml*, n = 10). It was significantly inhibited by phenylbutazone (oedema volume, 0.4 ± 0.07 *ml*, n=6; $P<0.05$) but not by any of the amino-acids. Nystatin-oedema was inhibited upto 6 hr by all aminoacids except L-leucine, which had some effect upto 2 hr only (Table II).

TABLE I : Effect of amino-acids and phenylbutazone on carrageenan-induced paw oedema in rats.

| *Test drug* | *Dose (mg/kg, p.o)* | *N* | *Oedema volume (ml)±S.E.M.* | *$ED_{50}$±95% C.L.* |
|---|---|---|---|---|
| Control | Nil | 10 | 0.72±0.10 | — |
| Phenylbutazone | 100 | 10 | 0.13±0.09*** | — |
| L-isoleucine | 50 | 6 | 0.54±0.17 | |
| | 100 | 6 | 0.48±0.10 | 182±0.18 |
| | 200 | 6 | 0.30±0.07** | (181.64 – 182.35) |
| | 280 | 6 | 0.28±0.09** | |
| DL-isoleucine | 50 | 6 | 0.60±0.23 | |
| | 100 | 6 | 0.52±0.11 | 218±0.28 |
| | 200 | 6 | 0.39±0.08* | (217.45 – 218.54) |
| | 400 | 6 | 0.30±0.07** | |
| L-leucine | 50 | 6 | 0.56±0.15 | |
| | 100 | 6 | 0.42±0.10* | 173±0.12 |
| | 200 | 6 | 0.26±0.08** | (172.76 – 173.23) |
| | 280 | 6 | 0.24±0.04*** | |
| DL-β-phenylalanine | 50 | 6 | 0.69±0.10 | |
| | 100 | 6 | 0.62±0.04 | 512±0.48 |
| | 399 | 6 | 0.44±0.09* | (511.06 – 512.94) |
| | 565 | 6 | 0.34±0.03** | |

*$P<0.05$ ; **$P<0.01$ ; ***$P<0.001$

**Granuloma pouch**

Phenylbutazone and the amino-acids except DL-β-phenylalanine caused significant reduction in granuloma pouch weight (Table III) ; the volume of exudate (2.3± 0.59 *ml*, n=10) was, however, not changed significantly by any drug. None of the rats used in this test showed the presence of the gastric ulcers. There was no significant change in the weights of the adrenal glands (35.5±2.2 *mg*, n=6) after the treatment with any drug.

**Castor oil diarrhoea**

Phenylbutazone and all the amino-acids inhibited castor oil induced diarrhoea significantly (Table III).

TABLE II : Effect of phenylbutazone and amino-acids on 5HT- and nystatin-induced pedal oedema in rats.

| *Test drug and dose* (*mg/kg in parentheses*) | *Mean oedema volume (ml) ±S.E.M.* | | | | | |
|---|---|---|---|---|---|---|
| | 5-HT *oedema* | | | *Nystatin oedema* | | |
| | *N* | *1½ hr* | *4 hr* | *N* | *2 hr* | *6 hr* |
| Controls – untreated | 10 | 0.56±0.10 | 0.66±0.12 | 10 | 0.28±0.01 | 0.61±0.01 |
| Phenylbutazone (100) | 10 | 0.16±0.08** | 0.21±0.06** | 10 | 0.10±0.08* | 0.24±0.08* |
| L-isoleucine (182) | 6 | 0.23±0.09* | 0.29±0.04** | 6 | 0.15±0.06* | 0.32±0.01* |
| DL-isoleucine (218) | 6 | 0.17±0.07** | 0.24±0.09* | 6 | 0.13±0.07* | 0.33±0.06* |
| L-leucine (173) | 6 | 0.20±0.04* | 0.23±0.04** | 6 | 0.11±0.07* | 0.39±0.16 |
| DL-β-phenylalanine (512) | 6 | 0.50±0.05 | 0.59±0.08 | 6 | 0.13±0.06* | 0.30±0.08* |

All drugs were given p o as suspension 30 min before the test. N=number of animals in the group.

*P<0.05; **P<0.01

TABLE III : Effect of amino acids in granuloma pouch test and castor oil diarrhoea test in rats.

| *Test drug and dose* (*mg/kg in parentheses*) | *Granuloma pouch* | *Castor oil diarrhoea* |
|---|---|---|
| | *Weight of granulation tissue mg ±S.E.M.* | *Mean number of diarrhoeal droppings at 3 hr (±S.E.M.) : Figures in parentheses indicate % inhibition in diarrhoeal droppings* |
| Control - untreated | 784±132 | 5.7±0.2 |
| Phenylbutazone (100) | 250±54** | 2.3±0.3*** (59.6) |
| L-isoleucine (182) | 508±11.4* | 4.0±0.5*** (28.1) |
| DL-isoleucine (218) | 466±81.1* | 4.5±0.2*** (22.8) |
| L-leucine (173) | 487±51* | 4.3±0.7* (24.5) |
| DL-β-phenylalanine (512) | 609±60.6 | 3.8±0.3*** (33.3) |

All drugs were given p o as suspension 30 min before the test. N (number of animals in the group)=6.

*P<0.05; **P<0.01; ***P<0.001

**Analgesia**

Phenylbutazone and L-isoleucine showed significant analgesic action which lasted upto 4 hr. DL-isoleucine had significant analgesic effect at ½ hr only while

L-leucine and DL-β-phenylalanine did not have any significant analgesic activity at any time (Table IV).

**Toxicity studies**

The amino-acids (upto 2 *g/kg*) given once (p o) showed no overt toxic symptoms or mortality in 24 hr. Findings in autopsies performed after 4 weeks also revealed no abnormalities.

TABLE IV : Analgesic effect of amino-acids and phenylbutazone as evaluated by hot wire technique.

| *Test drug and dose (mg/kg in parentheses)* | *Reaction time before treatment in sec mean ±S.E.M.* | *Reaction time after drug treatment in sec. mean±S.E.M.* | | | |
|---|---|---|---|---|---|
| | | *½ hr* | *1 hr* | *2 hr* | *4 hr* |
| Control – untreated | 5.9±0.4 | 5.8±0.9 | 6.2±0.5 | 6.2±1.3 | 6.0±1.0 |
| Phenylbutazone (100) | 5.7±0.4 | 8.8±0.8** | 8.9±0.2* | 11.2±0.9* | 11.1±2.7*** |
| L-isoleucine (182) | 4.2±0.3 | 7.0±0.6* | 8.2±0.8* | 8.6±1.0* | 9.0±1.9*** |
| DL-isoleucine (218) | 5.3±0.5 | 6.8±0.5*** | 7.2±0.96 | 8.0±2.0 | 8.0±2.4 |
| L-leucine (173) | 6.5±0.4 | 6.3±0.3 | 6.9±1.6 | 6.8±0.8 | 6.5±1.2 |
| DL-β-phenylalanine (512) | 5.8±0.5 | 7.2±1.0 | 8.0±0.8*** | 8.4±2.9 | 9.8±3.6 |

All drugs were given p o as suspension 30 min before the test. N (number of animals in the group) = 6.

*P<0.001; **P<0.01; ***P<0.05

## DISCUSSION

Carrageenan-induced oedema is reportedly mediated through release of 5-HT, histamine and prostaglandins (7,8,27). It is possible that the amino-acids studied here inhibit carrageenan-induced oedema by antagonizing effect of these mediators as also suggested for other amino-acids (11-15, 18,19,21) though DL-β-phenylalanine was not effective against 5-HT-oedema. *In vivo* the propulsion in intestines is activated by castor-oil and this may well be associated with a nonspecific increment in prostaglandin biosynthesis (24). Our results in castor-oil diarrhoea test suggest that the amino-acids and phenylbutazone are similar in action to aspirin like compounds which have been shown to cause a small but significant delay of castor oil-induced intestinal evacuation due to inhibition of prostaglandin biosynthesis (3).

Nystatin induces inflammatory reaction by labilizing the lysosomal membranes and this reaction is inhibited by both steroidal and non-steroidal anti-phlogistic drugs

(2), which have been shown to have stabilizing effects on lysosomal membranes both *in vivo* and *in vitro* (16,23). In the present investigation phenylbutazone and all the amino-acids caused significant inhibition of nystatin-induced oedema and like other amino-acids (11-15, 18,19,21) this effect is being possibly due to stabilization of the lysosomal membranes. It has been suggested that by stabilizing the lysosomal membrane the anti-inflammatory drugs may cause an interference with the *de nove* synthesis of enzymes (2), which is considered to be the final common pathway in inflammation (23,29). We suggest that like other anti-inflammatory drugs these amino-acids reduce inflammation by suppressing lysosomal phospholipase (7,27), which is responsible for formation of prostaglandins at the site of inflammation (1).

In order to evaluate the effect of amino-acids on the proliferative phase of inflammation the formaldehyde-induced inflammation and granuloma pouch techniques were employed. Like indomethacin and flufenamic acid (28) the amino-acids studied did not significantly inhibit the formaldehyde induced arthritis. Hence they differ from the other amino-acids which have been reported to significantly inhibit the formaldehyde induced arthritis (11-15, 18,19,21). However, all the amino-acids except DL-β-phenylalanine significantly decreased the weight of the granulation tissue. Their inhibitory effect on exudate formation was not significant. Steroidal anti-inflammatory agents suppress both exudate as well as granuloma formation (25) while non-steroidal anti-inflammatory agents cause inhibition of granulation tissue only (9). Consequently it can be deduced that amino-acids resemble the later group of drugs. Further, since there was no significant alteration in the weight of the adrenal glands these amino-acids do not apparently stimulate pituitary-adrenal system.

Phenylbutazone, L-isoleucine and DL-isoleucine showed significant analgesic activity while L-leucine and DL-β-phenylalanine did not show this action. Lack of gastric irritation in effective doses, absence of acute toxicity and presence of anti-inflammatory activity which compares favourably with phenylbutazone indicate that L-isoleucine, DL-isoleucine and L-leucine merit further studies.

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