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ANTI-INFLAMMATORY ACTIVITY OF THIAMINE AND NICOTINIC ACID

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Abstract—The anti-inflammatory properties of nicotinic acid and thiamine were investigated in carrageenin edema, formaldehyde edema and cotton pellet granuloma in male albino rats. The anti-inflammatory properties of these vitamins have been compared with those of phenylbutazone, a nonsteroidal anti-inflammatory drug. Nicotinic acid and thiamine have shown potent anti-inflammatory action in carrageenin edema, as well as in formaldehyde edema, as compared with phenylbutazone. Thiamine failed to show anti-inflammatory action in cotton pellet granuloma. The activities of liver glutamic oxaloacetic transaminase (GOT) and glutamic transaminase (GPT) increased in all types of inflammations, with the greatest increase in formaldehyde granuloma and the least in cotton pellet granuloma. Like phenylbutazone, nicotinic acid and thiamine inhibited both GPT and GOT activities in carrageenin edema, formaldehyde edema and cotton pellet granuloma. Nicotinic acid and thiamine also inhibited liver GPT in normal rats. The significance of the anti-inflammatory properties of nicotinic acid and thiamine is discussed.

VITAMINS are vital factors which help to restore to normal the functional changes occurring in deficiency states. However, there is considerable evidence that vitamins used in larger doses can also exert therapeutic or pharmacological effects under certain conditions.[1]

Ascorbic acid plays a vital role in the healing of wounds and fractures.[2-6] The role of vitamin K and its analogues as anti-inflammatory agents in certain chronic types of inflammation such as cotton pellet granuloma in rats has been reported.[6,7]

The present investigation was carried out to study the possible role of thiamine and nicotinic acid in the process of inflammation. The effects of these vitamins were studied on carrageenin- and formalin-induced edemas as well as on cotton pellet granuloma in rats. In addition, the effects of these agents on liver transaminases during inflammation were also investigated. Phenylbutazone, a nonsteroidal anti-inflammatory drug, was used as standard for comparison.

METHODS AND MATERIALS

Male albino rats (Haffkine Institute), weighing 80–120 g, were used for the study. Thiamine and nicotinic acid were dissolved in distilled water and administered intraperitoneally.

Carrageenin edema test. Edema was produced acutely by injecting the phlogistic agent, calcium carrageenin, into the plantar region of the hind paws of rats according to the method of Winter *et al.*[8]

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The rats were divided into four groups; three of these groups served as test groups, each receiving one of the drugs, nicotinic acid (30 mg), thiamine (30 mg) or phenylbutazone (10 mg) intraperitoneally. The fourth group received 1·0 ml of the vehicle per 100 g body wt. After 1 hr, 0·1 ml of 1 per cent (w/v) calcium carrageenin was injected into the plantar region of each hind paw of the rat. The volume changes in the rat paws were measured using a plethysmographic apparatus both initially and 3 hr after the injection of the carrageenin. After measuring inflammation, the rats were sacrificed and the liver was removed for biochemical studies.

Formalin edema test. Formalin edema was produced in rats by the method of Brownlee.[9] Rats were divided into four groups as previously described and nicotinic acid (30 mg), thiamine (30 mg) or phenylbutazone (10 mg) was injected intraperitoneally (i.p.) each day for 9 days. The control group received vehicle, 1·0 ml/100 g body wt. i.p., for the same period. On the tenth day, the rats were sacrificed and the liver was removed for biochemical studies. The 10-day average volume changes in rat paws were measured with the plethysmographic apparatus before sacrifice.

Cotton pellet granuloma test. Cotton pellet granuloma was produced in rats by a modification of the method of Winter and Porter.[10] The pellets, weighing exactly 10 mg each, were made with 5 mm sections of cotton rolls. The cotton pellets were sterilized in an autoclave for 30–45 min under 15 pounds of pressure. Four pellets were inserted subcutaneously in the ventral region, two on each side, in each rat under light ether anesthesia.

Rats received either nicotinic acid (30 mg), thiamine (30 mg) or phenylbutazone (10 mg) i.p. daily for 7 days. On the eighth day, the animals were sacrificed and the cotton pellets and liver were quickly removed.

The cotton pellets were freed from extraneous tissue and weighed. The granulomas were then dried in a hot-air oven at 70° to constant weight.

Plasma dye test. The rats were divided into four groups as previously described. The dye, Evans blue (0·4 per cent), was prepared in normal saline and given i.v. (0·5 ml/100 g) at the same time the drugs (thiamine, 30 mg; nicotinic acid, 30 mg; or phenylbutazone, 10 mg) were injected i.p. and carrageenin was given subcutaneously in the plantar region of the hind paws. The plasma Evans blue concentration was determined according to Young[11] 4 hr after the injection of carrageenin.

Biochemical parameters. Glutamic pyruvic transaminases (GPT) and glutamic oxaloacetic transaminases (GOT) were assayed in 0·5 per cent (w/v) liver homogenates by the method of Reitman and Frankel.[12] (One unit of enzyme activity was the change in optical density of 0·001/min/5 mg of liver tissue as measured with a Bausch & Lomb spectronic 20 colorimeter.) All the results were expressed in terms of wet weight of tissues.

RESULTS

Effect of vitamins on carrageenin edema. The results in Table 1 indicate that nicotinic acid and thiamine reduced edema significantly. When compared with phenylbutazone (63 per cent), nicotinic acid (77 per cent) and thiamine (68 per cent) showed comparable degrees of anti-inflammatory activity.

Effect of nicotinic acid and thiamine on formaldehyde edema. Nicotinic acid and thiamine showed anti-inflammatory activity similar to that of phenylbutazone (42 per

TABLE 1. EFFECT OF NICOTINIC ACID AND THIAMINE ON CARRAGEENIN-INDUCED EDEMA

Drugs	No. of rats	Dose (mg/rat, i.p.)	Edema (Mean ± S.E.)*	Inhibition (%)
Control	15		60 ± 2·1	
Phenylbutazone	6	10	22 ± 2·3 ($P < 0·001$)	63
Nicotinic acid	7	30	14 ± 0·63 ($P < 0·001$)	77
Thiamine	7	30	19 ± 3·4 ($P < 0·001$)	68

* Results are expressed as mean ± S.E. of manometer readings. The manometer's 100 divisions correspond to 13·6 g mercury.

cent). Quantitatively, nicotinic acid (47 per cent) had more potent anti-inflammatory activity when compared with thiamine (34 per cent; Table 2).

TABLE 2. EFFECT OF NICOTINIC ACID AND THIAMINE ON FORMALIN-INDUCED EDEMA

Drugs	No. of rats	Dose (mg/rat, i.p.)	Average edema ± S.E.* (10 days)	Inhibition (%)
Control	10		38 ± 1·92	
Phenylbutazone	8	10	22 ± 1·4 ($P < 0·001$)	42
Nicotinic acid	10	30	20 ± 1·8 ($P < 0·001$)	47
Thiamine	10	30	25 ± 1·8 ($P < 0·001$)	34

* Results are expressed as mean ± S.E. of manometer readings. The manometer's 100 divisions correspond to 13·6 g mercury.

Effect of nicotinic acid and thiamine on cotton pellet granuloma. Nicotinic acid produced marked granuloma inhibition (62 per cent) and was more potent when compared with phenylbutazone (49 per cent). Thiamine failed to inhibit granuloma formation significantly (Table 3).

TABLE 3. EFFECT OF NICOTINIC ACID AND THIAMINE ON COTTON PELLET GRANULOMA

Drug	No. of rats	Dose (mg/rat, i.p.)	Dry wt. of granuloma ± S.E. (mg)	Granuloma inhibition (%)
Control	10		204 ± 2·6	
Phenylbutazone	8	10	104 ± 6·8 ($P < 0·001$)	49
Nicotinic acid	8	30	78 ± 3·5 ($P < 0·001$)	62
Thiamine	7	30	190 ± 9·8 ($P > 0·6$)	7

Effect of nicotinic acid and thiamine on plasma dye. Thiamine and phenylbutazone increased plasma Evans blue concentration by 6 and 4 per cent respectively. Nicotinic acid decreased plasma Evans blue concentration by 4 per cent (Table 4).

TABLE 4. EFFECT OF NICOTINIC ACID AND THIAMINE ON PLASMA EVANS BLUE IN CARRAGEENIN-INDUCED INFLAMMATION

Drugs	Dose (mg/rat, i.p.)	No. of rats	Plasma Evans blue concn. (% change)
Distilled water	1 ml	10	0
Nicotinic acid	30	10	− 4
Thiamine	30	10	+ 6
Phenylbutazone	10	10	+ 4

Effect of nicotinic acid and thiamine on liver GPT and GOT in carrageenin-induced edema. It was observed that the activities of both GPT and GOT of liver were significantly elevated during inflammation (edema) produced by carrageenin. The elevated activities of these enzymes were significantly inhibited by nicotinic acid, thiamine and phenylbutazone (Table 5).

TABLE 5. EFFECT OF NICOTINIC ACID AND THIAMINE ON LIVER GLUTAMIC PYRUVIC TRANSAMINASE (GPT) AND GLUTAMIC OXALOACETIC TRANSAMINASE (GOT) IN CARRAGEENIN EDEMA*

	No. of rats	Control (12)	Nicotinic acid (8)	Thiamine (8)	Phenylbutazone (8)
Liver GPT†	Normal	18·8 ± 1·5	12·9 ± 1·2 (P < 0·001)	13·4 ± 0·44 (P < 0·001)	13·5 ± 0·82 (P < 0·001)
	% Decrease with drug		31·4	28·4	28
	Carrageenin edema	26·4 ± 1·9	16·5 ± 0·56 (P < 0·001)	13·2 ± 2·5 (P < 0·001)	12·3 ± 0·46 (P < 0·001)
	% Decrease with drug		37·5	50	54
Liver GOT†	Normal	21·5 ± 1·37	19·40 ± 1·5 (P > 0·3)	20·4 ± 0·44 (P > 0·6)	19·2 ± 1·3 (P > 0·2)
	% Decrease with drug		10‡	5‡	11‡
	Carrageenin edema	32·7 ± 1·1	16·65 ± 0·9 (P < 0·001)	19·1 ± 1·8 (P < 0·001)	13·0 ± 1·2 (P < 0·001)
	% Decrease with drug	49	49	41	60

* The results are expressed as mean ± S.E. of enzyme activity.
† Enzyme activity in units $\times 10^3$/g of liver; one unit = change in o.d. of 0·001/min/5 mg liver tissue.
‡ Not significant.

Effect of nicotinic acid and thiamine on liver GPT and GOT in formalin-induced edema. Nicotinic acid, thiamine and phenylbutazone markedly inhibited the activities of GPT

and GOT in formalin edema. The GPT and GOT activities induced by formalin edema increased more markedly during inflammation than did those produced by carrageenin edema (Table 6).

TABLE 6. EFFECT OF NICOTINIC ACID AND THIAMINE ON LIVER GLUTAMIC PYRUVIC TRANSAMINASE (GPT) AND GLUTAMIC OXALOACETIC TRANSAMINASE (GOT) IN FORMALIN-INDUCED EDEMA*

	No. of rats	Control (12)	Nicotinic acid (7)	Thiamine (6)	Phenylbutazone (8)
	Normal	18·8 ± 1·5	12·4 ± 1·1 (P < 0·001)	12·5 ± 0·44 (P < 0·001)	12·8 ± 0·84 (P < 0·001)
	% Decrease with drug		33	33	33
Liver GPT†	Formalin edema	30·76 ± 2·0	17·74 ± 0·94 (P < 0·001)	19·5 ± 0·85 (P < 0·001)	16·8 ± 0·88 (P < 0·001)
	% Decrease with drug		42	36	45·4
Liver GOT†	Normal	21·5 ± 1·37	19·8 ± 2·2 (P > 0·4)	20·2 ± 2·3 (P > 0·6)	20·6 ± 1·2 (P > 0·7)
	% Decrease with drug		8‡	6‡	4‡
	Formalin edema	38·9 ± 2·55	21·9 ± 1·6 (P < 0·001)	24·0 ± 1·3 (P < 0·001)	20·2 ± 1·6 (P < 0·001)
	% Decrease with drug		44	38	48·7

* The results are expressed as mean ± S.E.
† Enzyme activity in units $\times 10^3$/g of liver; one unit = change in O. D. of 0·001/min/5 mg liver tissue.
‡ Not significant.

Effect of nicotinic acid and thiamine on liver GPT and GOT in cotton pellet granuloma. Nicotinic acid, thiamine and phenylbutazone inhibited significantly the activities of GPT and GOT in liver in cotton pellet granuloma. No significant increase in GOT and GPT activities was produced during inflammation by cotton pellet granuloma (Table 7).

In all of the groups, liver GPT activity was significantly inhibited by nicotinic acid, thiamine and phenylbutazone (Tables 5–7).

DISCUSSION

Thiamine and nicotinic acid showed significant anti-inflammatory action in the various types of inflammation used in the present studies. Anti-inflammatory effects of these vitamins are nearly of the same order as that of phenylbutazone, except in the case of thiamine, which did not show significant anti-inflammatory activity in the cotton pellet granuloma test.

The findings were supported by a significant inhibition by these vitamins of liver glutamic oxaloacetic transaminases, a characteristic of anti-inflammatory action.[13–16]

It is well known that both thiamine and nicotinic acid exert various therapeutic and pharmacological effects in conditions other than deficiency states. Thiamine is an

TABLE 7. EFFECT OF NICOTINIC ACID AND THIAMINE ON LIVER GLUTAMIC PYRUVIC TRANSAMINASE (GPT) AND GLUTAMIC OXALOACETIC TRANSAMINASE (GOT) IN COTTON PELLET GRANULOMA*

	No. of rats	Control (12)	Nicotinic acid (7)	Thiamine (7)	Phenylbutazone (7)
	Normal	18·8 ± 1·5	13·1 ± 1·2 (P < 0·01)	12·8 ± 0·64 (P < 0·001)	11·8 ± 0·72 (P < 0·001)
	% Decrease with drug		29	32	37·0
Liver GPT†	Cotton pellet granuloma	21·4 ± 2·2	9·9 ± 1·3 (P < 0·001)	12·0 ± 1·9 (P < 0·001)	8·2 ± 1·1 (P < 0·001)
	% Decrease with drug		54	44	62
Liver GOT†	Normal	21·5 ± 1·47	20·8 ± 1·5 (P > 0·4)	19·8 ± 1·2 (P > 0·4)	20·6 ± 1·2 (P > 0·4)
	% Decrease with drug		13‡	8‡	4‡
	Cotton pellet granuloma	25·2 ± 2·2	17·1 ± 1·1 (P < 0·001)	19·2 ± 1·3 (P < 0·01)	16·2 ± 1·3 (P < 0·001)
	% Decrease with drug		32	24	36

* The results are expressed as mean ± S.E.

† Enzyme activity in units $\times 10^3$/g of liver; one unit = change in O. D. of 0·001/min/5 mg of liver tissue.

‡ Not significant.

essential factor in the transmission of nerve impulses[17] and clinically is used in the treatment of peripheral neuritis and polyneuritis.[18] It is also claimed that thiamine has beneficial effects on diabetes mellitus,[19] gout[19] and hyperthyroidism.[20]

The vasodilating property of nicotinic acid is well known and has found wide application in a number of diseases such as Meniere's syndrome,[21] perennial vasomotor rhinitis[22] and angina pectoris.[23] Pronounced anti-inflammatory action of sodium nicotinate in aseptic inflammation in rabbits has been reported.[24] The anti-inflammatory actions of thiamine and nicotinic acid in our experiments may be responsible for some of the beneficial effects of these vitamins observed in various conditions of neuritis, even though there may not be a physiological deficiency of these vitamins.

Many nonspecific effects can be obtained in experiments on the inhibition of foot edema in rats, especially when drugs are administered by the intraperitoneal route.[25] They may irritate the peritoneal surface or produce permeability changes in peritoneal membranes.[25] Our experiments with plasma dye indicate that the anti-inflammatory effect of nicotinic acid and thiamine is specific and not due to permeability changes in peritoneal membranes. The inhibition of transaminases by these vitamins in different types of inflammation supports their anti-inflammatory activities.

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REFERENCES

1. H. Molitor and G. A. Emerson, *Vitams. Horm.* **6**, 69 (1948).
2. T. H. Lanman and T. H. Ingalls, *Ann. Surg.* **105**, 616 (1937).
3. M. K. Bartlett, C. M. Jones and A. E. Ryan, *Ann. Surg.* **111**, 1 (1940).
4. G. H. Bourne, *Lancet* **1**, 688 (1944).
5. H. J. Lauber, H. Nafziger and T. Bersin, *Klin. Wschr.* **16**, 1313 (1937).
6. H. Ibayashi, T. Yamaji, T. Tajima and K. Nakao, *Endocrinology* **76**, 780 (1965).
7. I. B. Kovacs, P. Gorog, L. Szporny and G. Fekete, *Biochem. Pharmac.* **16**, 575 (1967).
8. C. A. Winter, E. A. Risley and G. W. Nuss, *Proc. Soc. exp. Biol. Med.* **111**, 544 (1962).
9. G. Brownlee, *Lancet* **1**, 157 (1950).
10. C. A. Winter and C. C. Porter, *J. Am. pharm. Ass.* **46**, 515 (1957).
11. D. A. B. Young, *Proc. Soc. exp. Biol. Med.* **116**, 220 (1964).
12. S. Reitman and S. Frankel, *Am. J. clin. Path.* **28**, 56 (1957).
13. K. K. Tangri, P. K. Seth, S. S. Parmar and K. P. Bhargava, *Biochem. Pharmac.* **14**, 1277 (1965).
14. P. K. Sheth and K. K. Tangri, *J. Pharm. Pharmac.* **18**, 831 (1966).
15. V. Moses and M. J. H. Smith, *Biochem. J.* **78**, 424 (1961).
16. S. G. Kalyanpur, S. Pohujani, S. R. Naik and U. K. Sheth, *Biochem. Pharmac.* **17**, 797 (1968).
17. E. Abderhalden and R. Abderhalden, *Klin. Wschr.* **17**, 1480 (1938).
18. L. Kalaja, *Acta med. scand.* **107**, 427 (1941).
19. M. G. Vorhaus, R. R. Williams and R. E. Waterman, *J. Am. med. Ass.* **105**, 1580 (1935).
20. R. H. Williams, E. Egana, P. Robinson, S. P. Asper and C. Dutott, *Archs intern. Med.* **72**, 353 (1943).
21. M. Atkinson, *J. Am. med. Ass.* **116**, 1753 (1941).
22. H. L. Williams, *Proc. Staff Meet. Mayo Clin.* **21**, 58 (1946).
23. F. J. Neuwahl, *Lancet* **2**, 419 (1942).
24. E. M. Chukichev, *Patol. Fiziol. eksp. Terap.* **3**, 39 (1959).
25. S. Garattini, A. Jori, D. Bernardi, C. Carrara, S. Paglialunga and D. Segre, in *Non-Steroidal Anti-inflammatory Drugs*, p. 151. Excerpta Med. Found. Int. Congr. Series, No. 82 (1965).