Biochemical Pharmacology, Vol. 17, pp. 797–803. Pergamon Press. 1968. Printed in Great Britain

STUDY OF BIOCHEMICAL EFFECTS OF ANTI-INFLAMMATORY DRUGS IN CARRAGEENIN INDUCED OEDEMA AND COTTON PELLET GRANULOMA

S. G. KALYANPUR, SAVITRI POHUJANI, S. R. NAIK and U. K. SHETH*

C.S.I.R. Pharmacology Research Unit, Department of Pharmacology,
G.S. Medical College, Parel, Bombay-12, India

(*Received* 27 *June* 1967; *accepted* 7 *December* 1967)

Abstract—The biochemical effects of anti-inflammatory drugs, hydrocortisone, phenylbutazone and indomethacin in carrageenin oedema and cotton pellet granuloma have been compared in male albino rats. In carrageenin oedema, the drugs had no effect upon ATPase activities of liver, brain or oedema tissue. In cotton pellet granuloma, the drugs significantly increased ATPase activity of granuloma tissue but not the activities of liver and brain. The ATPase activities of liver and brain remained unaltered during carrageenin oedema as well as cotton pellet granuloma.

The activities of liver transaminases, glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) increased more significantly in carrageenin oedema than in cotton pellet granuloma. Hydrocortisone, phenylbutazone and indomethacin significantly inhibited both GPT and GOT activities in liver in carrageenin oedema and cotton pellet granuloma. The drugs also inhibited liver GPT in normal rats.

The significance of these biochemical effects is discussed in relation to the pharmacological activities of the drugs.

INFLAMMATION produced by various phlogistic agents (irritants) has been used as a standard procedure for screening anti-inflammatory drugs. A number of phlogistic agents of diverse nature such as formalin, yeast, dextran, egg white and carrageenin have been employed to test the anti-inflammatory activity of various drugs.[1–4] Inhibition of cotton pellet granuloma in rats has also been used as a measure of anti-inflammatory activity.[5, 6]

It has been shown that different types of paw oedema in rats showed varied responses to various anti-inflammatory drugs, each drug showing a different profile of activity against different type of oedema.[7, 8] However a fairly good correlation was also observed between the responses of carrageenin oedema and cotton pellet granuloma to various anti-inflammatory drugs.[7, 8]

It has been reported that anti-inflammatory drugs exert a number of biochemical effects such as uncoupling of oxidative phosphorylation,[9–11] stimulation of adenosine triphosphatase,[12–15] and inhibition of transaminases.[13–15] It seemed interesting to see whether the biochemical changes produced during different types of inflammations are different and how these changes are affected by various anti-inflammatory drugs. In the present investigation effects of anti-inflammatory drugs, hydrocortisone,

* Professor of Pharmacology.

phenylbutazone and indomethacin on the biochemical parameters, adenosinetriphosphatase (ATPase) and transaminases were compared in carrageenin oedema and cotton pellet granuloma.

METHODS AND MATERIALS

Male albino rats weighing 80–100 g were used throughout this study.

The anti-inflammatory drugs, hydrocortisone, phenylbutazone and indomethacin were administered orally as uniform suspensions in 1% (w/v) carboxymethyl cellulose (CMC).

Carrageenin oedema test. Oedema was produced acutely by injection of a phlogistic agent, carrageenin in the plantar region of the hind paws of the rat according to the method of Winter *et al.*[4]

The drugs, hydrocortisone (80 mg/kg), phenylbutazone (200 mg/kg) and indomethacin (10 mg/kg) were administered orally to rats in a volume of 1·0 ml/100 g body wt., followed by 4·0 ml of distilled water. The latter treatment seemed necessary to insure uniform hydration of the animals and to reduce the variability of oedema formation. The control animals received 1·0 ml of the vehicle/100 g body wt. followed by 4·0 ml of distilled water. After exactly 1 hr, 0·1 ml of 1% (w/v) carrageenin was injected in the plantar region of each hind paw of rat. The animals were sacrificed 3 hr after the administration of carrageenin. The oedema tissue from hind paws, liver and brain were quickly removed for biochemical studies.

Cotton pellet granuloma test. The cotton pellet granuloma was produced in rats by the method of Winter and Porter[5] with slight modifications. The pellets weighing exactly 10 mg each were made from 5 mm sections of cotton rolls. The cotton pellets were sterilized in an autoclave for 30–45 min under 15 lb pressure. Four pellets were inserted subcutaneously in the ventral region, two on either side, in each rat under light ether anaesthesia. The drugs, hydrocortisone (10 mg/kg), phenylbutazone (100 mg/kg), and indomethacin (5 mg/kg) were administered orally to rats in a volume of 1·0 ml/100 g body wt. daily for 7 days. Controls received 1·0 ml vehicle/100 g body wt. for the same period. On the eighth day the animals were sacrificed, the granuloma tissue (along with cotton pellets), liver and brain were quickly removed.

Study of biochemical parameters

The adenosinetriphosphatase (ATPase) activity was measured in homogenates of oedema tissue, granuloma tissue (separated from cotton pellets), liver and brain, prepared in 0·25 M sucrose in Potter–Elvehjem type glass homogenizer. 5% (w/v) homogenates were made from the oedema tissue and granuloma tissue while 10% (w/v) homogenates were prepared from liver and brain.

The complete assay system consisted of 0·05 M Tris buffer; pH 8·0, 1 mM ATP, 10 mM $MgCl_2$ and 0·2 ml tissue homogenate, in a final volume of 2·0 ml. After incubation at 37° for 30 min the reaction was stopped by addition of 1·3 ml of 30% (w/v) trichloroacetic acid. A suitable aliquot of the clear supernatant after centrifugation was used for the determination of Pi (inorganic phosphorus) released from ATP by the method of Taussky and Shorr.[16] One unit of enzyme activity was equivalent to μM of Pi/100 mg tissue/30 min at 37°.

Transaminases, glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) were assayed in 0·5% (w/v) liver homogenates by the method of Reitman and Frankel.[17] One unit of enzyme activity was the change in the optical density of 0·001/min/5 mg liver tissue.

Bausch and Lomb Spectronic '20' colorimeter was used for the measurements of optical density.

All results were expressed in terms of wet weight of tissues.

RESULTS

Effect on adenosinetriphosphatase (ATPase) activity in carrageenin oedema

The results in Table 1 show that none of the anti-inflammatory drugs had any appreciable effect on ATPase activity in liver, brain or oedema tissue itself. Even the most potent drug, indomethacin (10 mg/kg) failed to show any effect on ATPase activity. The drugs had no effect on ATPase activities of liver and brain in normal rats. It was also interesting to note that the ATPase activities of liver and brain remained unaltered during carrageenin oedema.

TABLE 1. EFFECT OF HYDROCORTISONE, PHENYLBUTAZONE AND INDOMETHACIN ON THE ADENOSINE TRIPHOSPHATASE (ATPASE) ACTIVITIES IN CARRAGEENIN OEDEMA

		Control	Hydrocortisone (80 mg/kg)	Phenylbutazone (200 mg/kg)	Indomethacin (10 mg/kg)
Liver ATPase*	No. of rats	(10)	(6)	(6)	(6)
	Normal	11·20 ± 0·45	11·10 ± 0·93	11·60 ± 0·45	10·70 ± 0·51
	% Increase with drug	—	—	—	—
	Carrageenin Oedema	11·16 ± 0·33	11·53 ± 0·61	11·31 ± 0·95	10·50 ± 0·45
	% Increase with drug	—	—	—	—
Brain ATPase*	Normal	9·70 ± 0·34	9·66 ± 0·62	10·00 ± 0·49	9·50 ± 0·23
	% Increase with drug	—	—	—	—
	Carrageenin Oedema	9·66 ± 0·32	10·00 ± 0·95	9·70 ± 0·86	9·00 ± 0·29
	% Increase with drug	—	—	—	—
Oedema tissue ATPase*	Carrageenin Oedema	5·15 ± 0·09	5·66 ± 0·65	5·12 ± 0·06	5·03 ± 0·19
	% Increase with drug	—	9·9	—	—

The results are expressed as Mean ± S.E.
* The ATPase activity is expressed as μM'Pi'/100 mg tissue/30 min at 37°.

Effect on adenoinsetriphosphatase (ATPase) activity in cotton pellet granuloma

The results in Table 2 indicate that all the drugs tested significantly increased ATPase activity of the cotton pellet granuloma tissue, while having no effect upon ATPase activities of liver and brain. Indomethacin (5 mg/kg) showed the maximum effect, increasing the ATPase activity by 74·6 per cent. The ATPase activities of liver and brain remained unaltered during inflammation while the activities in normal rats were unaffected by the drugs.

TABLE 2. EFFECT OF HYDROCORTISONE, PHENYLBUTAZONE AND INDOMETHACIN ON ADENOSINE TRIPHOSPHATASE (ATPASE) ACTIVITIES IN COTTON PELLET GRANULOMA

		Control	Hydrocortisone (10 mg/kg)	Phenylbutazone (100 mg/kg)	Indomethacin (5 mg/kg)
Liver ATPase*	No. of rats	(6)	(5)	(5)	(5)
	Normal	11·20 ± 0·45	11·20 ± 0·44	11·40 ± 0·18	11·60 ± 0·46
	% Increase with drug	—	—	—	—
	Cotton pellet granuloma	10·12 ± 1·04	9·80 ± 0·11	10·64 ± 0·59	10·58 ± 0·46
	% Increase with drug	—	—	—	—
Brain ATPase*	Normal	9·70 ± 0·34	9·64 ± 0·54	10·00 ± 0·45	9·92 ± 0·90
	% Increase with drug	—	—	—	—
	Cotton pellet granuloma	9·02 ± 0·02	8·84 ± 0·03	9·44 ± 0·55	9·35 ± 0·22
	% Increase with drug	—	—	—	—
Granuloma tissue ATPase*	Cotton pellet granuloma	5·20 ± 0·29	6·41 ± 0·27	7·70 ± 0·60	9·08 ± 0·13
	% Increase with drug	—	23·3 ($P < 0·02$)	48·0 ($P < 0·01$)	74·6 ($P < 0·001$)

The results are expressed as Mean ± S.E.
* The ATPase activity is expressed as μM of Pi,/100 mg tissue/30 min at 37°.

Effect on liver glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) in carrageenin oedema

The activities of both glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) in liver were significantly inhibited by all the drugs in carrageenin oedema (Table 3). Indomethacin (10 mg/kg) caused maximum inhibition of both the transaminases. It was observed that the activities of both GPT and GOT were significantly, elevated during inflammation (oedema) produced by carrageenin. It was also interesting to note that the liver glutamic-pyruvic transaminase was significantly inhibited in normal rats by all the drugs.

Effect on liver glutamic-pyruvic transminase (GPT) and glutamic-oxaloacetic transaminase (GOT) in cotton pellet granuloma

Results (Table 4) indicate that hydrocortisone (10 mg/kg) phenylbutazone (100 mg/kg) and indomethacin (5 mg/kg) markedly inhibited the activities of GPT and GOT in liver in cotton pellet granuloma. In contrast to carrageenin oedema the GPT and GOT activities increased only slightly during the inflammation induced by cotton pellet granuloma. The liver GPT activity in normal rats was significantly inhibited by all the drugs tested

DISCUSSION

The evidence from this study indicates that the anti-inflammatory drugs, hydrocortisone, phenylbutazone and indomethacin had practically no effect upon the adenosinetriphosphatase (ATPase) activity in carrageenin induced oedema. The drugs failed to show any effect on ATPase activity not only in liver and brain but also in the

TABLE 3. EFFECT OF HYDROCORTISONE, PHENYLBUTAZONE AND INDOMETHACIN ON LIVER GLUTAMIC PYRUVIC TRANSAMINASE (GPT) AND GLUTAMIC OXALOACETIC TRANSAMINASE (GOT) IN CARRAGEEN OEDEMA

		Control	Hydrocortisone (80 mg/kg)	Phenylbutazone (200 mg/kg)	Indomethacin (10 mg/kg)
	No. of rats	(10)	(5)	(5)	(5)
	Normal	15·30 ± 0·96	11·60 ± 1·1 ($P < 0·02$)	11·30 ± 1·55 ($P < 0·05$)	7·64 ± 1·03 ($P < 0·001$)
Liver GPT*	% Decrease with drug	—	24·2	26·0	50·0
	Carrageenin Oedema	28·08 ± 1·81	12·20 ± 0·62 ($P < 0·001$)	9·76 ± 1·70 ($P < 0·001$)	13·20 ± 1·55 ($P < 0·001$)
	% Decrease with drug	—	56·4	65·0	53·2
	Normal	19·00 ± 1·50	17·1 ± 1·30 ($P > 0·3$)	16·80 ± 0·40 ($P > 0·1$)	16·4 ± 0·62 ($P > 0·1$)
Liver GOT*	% Decrease with drug	—	10·0	11·5	13·6
	Carrageenin Oedema	35·92 ± 2·33	15·42 ± 1·29 ($P < 0·001$)	8·96 ± 0·57 ($P < 0·001$)	14·33 ± 1·68 ($P < 0·001$)
	% Decrease with drug	—	57·0	73·0	60·0

The results are expressed as Mean ± S.E.

* Enzyme activity in units × 10^3/g liver. One unit = change in O.D. of 0·001/min/5 mg liver tissue.

TABLE 4. EFFECT OF HYDROCORTISONE, PHENYLBUTAZONE AND INDOMETHACIN ON LIVER GLUTAMIC PYRUVIC TRANSAMINASE (GPT) AND GLUTAMIC OXALOACETIC TRANSAMINASE (GOT) IN COTTON PELLET GRANULOMA

		Control	Hydrocortisone (10 mg/kg)	Phenylbutazone (100 mg/kg)	Indomethacin (5 mg/kg)
	No. of rats	(5)	(5)	(5)	(5)
	Normal	15·30 ± 0·96	11·79 ± 1·50 ($P < 0·05$)	9·36 ± 0·80 ($P < 0·001$)	6·40 ± 0·02 ($P < 0·001$)
Liver GPT*	% Decrease with drug	—	23·5	38·8	57·5
	Cotton pellet granuloma	18·00 ± 1·90	13·36 ± 1·12 ($P = 0·05$)	5·04 ± 0·62 ($P < 0·001$)	3·76 ± 0·51 ($P < 0·001$)
	% Decrease with drug	—	26·0	72·0	79·0
	Normal	19·00 ± 1·50	17·50 ± 0·93 ($P = 0·4$)	16·60 ± 0·46 ($P > 0·1$)	16·0 ± 0·52 ($P > 0·1$)
	% Decrease with drug	—	8·0	12·6	15·7
Liver GOT*	Cotton pellet granuloma	22·12 ± 1·9	4·13 ± 0·70 ($P < 0·001$)	9·84 ± 1·14 ($P < 0·001$)	4·92 ± 0·55 ($P < 0·001$)
	% Decrease with drug	—	81·0	55·8	78·2

The results are expressed as Mean ± S.E.

* Enzyme activity in units × 10^3/g liver. One unit = change in O.D. of 0·001/min/5 mg liver tissue.

inflamed oedema tissue itself. Another interesting observation was that the ATPase activities of liver and brain remained unaltered during carrageenin oedema and that these activities in normal rats were unaffected by the anti-inflammatory drugs.

However it was observed that in cotton pellet granuloma the drugs significantly increased ATPase activity of granuloma tissue but not the activities of liver and brain. It was also interesting to note that as in carrageenin oedema, the ATPase activities of liver and brain remained unaltered in cotton pellet granuloma inflammation. The ATPase activities of liver and brain in normal rats were also not affected by the drugs.

Thus it was evident that the biochemical effects of hydrocortisone, phenylbutazone and indomethacin concerning ATPase activity were totally different in two types of inflammation, viz. carrageenin oedema and cotton pellet granuloma. It was somewhat an ambiguous finding that the drugs even in relatively high doses failed to affect the ATPase activity in an acute inflammation, such as carrageenin oedema while stimulating the enzyme activity in chronic sub-acute inflammation, cotton pellet granuloma. Presumably the drugs stimulate ATPase activity only after repeated administration as in cotton pellet granuloma. Such stimulation of ATPase activity by anti-inflammatory drugs after repeated administration has been reported in the case of formalin induced arthritis.[13, 14, 18] It has been suggested that the stimulatory effect of anti-inflammatory drugs on the ATPase activity may not be related to their anti-inflammatory action since the ATPase activity remained unaltered during inflammation.[13, 14, 18] This might partly explain the failure of anti-inflammatory drugs in our study, to stimulate ATPase activity in carrageenin oedema.

Our experiments show that the activities of transaminases, glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) in liver were increased in both carrageenin oedema and cotton pellet granuloma. The activities of GPT and GOT in liver increased significantly in carrageenin oedema but increased slightly in cotton pellet granuloma. The drugs, hydrocortisone, phenulbutazone and indomethacin significantly inhibited both GPT and GOT in liver in carrageenin oedema as well as in cotton pellet granuloma. Interesting to note was that the liver glutamic-pyruvic transaminase (GPT) was inhibited by the drugs in normal rats as well. It appears, therefore, that the inhibition of liver GPT may not be related to the anti-inflammatory action of drugs as suggested earlier.[13, 14, 18] On the other hand the drugs had no significant effect on liver GPT in normal rats. Therefore, the inhibition of the liver GOT activity may be related to the anti-inflammatory action of the drugs and this observation is supported well by previous findings.[13, 14, 18]

The comparison of the effects of three drugs tested, on ATPase activity of cotton pellet granuloma tissue, shows that indomethacin was the most potent stimulator of ATPase activity, followed by hydrocortisone and then phenylbutazone. In both carrageenin oedema and cotton pellet granuloma the inhibitory effects of the three drugs on liver GOT activity were of the same order; indomethacin being the most potent inhibitor followed by hydrocortisone and then phenylbutazone.

Winter *et al.*[6] have shown that the anti-inflammatory activities of hydrocortisone, phenylbutazone and indomethacin were of order: indomethacin > hydrocortisone > phenylbutazone, in both carrageenin oedema and cotton pellet granuloma. There is therefore, good agreement in this study, between the anti-inflammatory activities and the biochemical activities of hydrocortisone, phenylbutazone and indomethacin.

In fact a close agreement between the relative anti-inflammatory activities of various non-steroidal anti-inflammatory drugs and their relative potencies in uncoupling oxidative phosphorylation has been demonstrated.[19]

REFERENCES

1. R. DOMENJOZ, *Ann. N.Y. Acad. Sci.* **86**, 263 (1960).
2. D. LORENZ, *Arch. exp. Path. Pharmak.* **241**, 516 (1961).
3. C. V. WINDER, J. WAX and M. A. BEEN, *Archs int. Pharmacodyn. Ther.* **112**, 174 (1957).
4. C. A. WINTER, E. A. RISLEY and G. W. NUSS, *Proc. Soc. exp. Biol. Med.* **111**, 544 (1962).
5. C. A. WINTER and C. C. PORTER, *J. Am. Pharm. Ass. Sci. Ed.* **46**, 515 (1957).
6. C. A. WINTER, E. A. RISLEY and G. W. NUSS, *J. Pharmac. exp. Ther.* **141**, 369 1963).
7. C. A. WINTER, *Int. Symp. Non-steroidal Anti-inflammatory Drugs, Milan*, (Eds. S. GA RATINI MILAN and M. N. G. DUKES), p. 190, Oss. (1964).
8. C. A. WINTER, E. A. RISLEY and G. W. NUSS, *Fedn Proc.* **23**, 284 (1964).
9. S. W. TEFFERY and M. J. H. SMITH, *Biochem. J.* **72**, 462 (1959).
10. M. W. WHITEHOUSE, *J. Pharm. Pharmac.* **15**, 556 (1963).
11. M. W. WHITEHOUSE, *Biochem. Pharmac.* **13**, 319 (1964).
12. M. W. WHITEHOUSE and J. M. HASLAM, *Nature, Lond.* **196**, 1323 (1962).
13. K. K. TANGRI, P. K. SETH, S. S. PARMAR and K. P. BHARGAVA, *Biochem. Pharmac.* **14**, 1277 (1965).
14. P. K. SETH and K. K. TANGRI, *J. Pharm. Pharmac.* **18**, 831 (1966).
15. V. MOSES and M. J. H. SMITH, *Biochem. J.* **78**, 424 (1961).
16. H. H. TAUSSKY and E. SHORR, *J. biol. chem.* **202**, 675 (1953).
17. S. REITMAN and S. FRANKEL, *Am. J. Clin. Path.* **28**, 56 (1957).
18. K. K. TANGRI, P. R. SAXENA, P. K. SETH and K. P. BHARGAVA, *Biochem. Pharmac.* **15**, 825 (1966).
19. S. S. ADAMS and R. COBB, *Nature, Lond.* **181**, 773 (1958).