Biochemical Pharmacology, Vol. 21, pp. 511–516. Pergamon Press, 1972. Printed in Great Britain

EFFECTS OF ANTI-INFLAMMATORY DRUGS ON GLUTATHIONE LEVELS AND LIVER SUCCINIC DEHYDROGENASE ACTIVITY IN CARRAGEENIN EDEMA AND COTTON PELLET GRANULOMA IN RATS

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(*Received* 5 *January* 1971; *accepted* 6 *August* 1971)

Abstract—Effects of anti-inflammatory drugs, hydrocortisone, phenylbutazone and indomethacin, on blood and liver glutathione (GSH) levels and liver succinic dehydrogenase (SDH) in carrageenin edema and cotton pellet granuloma have been investigated in male albino rats. No significant changes in liver and blood GSH levels during carrageenin edema and cotton pellet granuloma were observed. Liver GSH levels were lowered significantly by these anti-inflammatory drugs in rats with both types of inflammation and also in normal rats. However, blood GSH levels were not altered significantly by these drugs in carrageenin-treated and normal rats except with indomethacin which lowered blood GSH levels both in normal and carrageenin-treated rats. These drugs lowered blood GSH both in rats with cotton pellet granuloma and in normal rats. Liver SDH activity was significantly increased in carrageenin edema and cotton pellet granuloma. Pretreatment with hydrocortisone, phenylbutazone, and indomethacin inhibited significantly the increased liver SDH activity in carrageenin edema and cotton pellet granuloma. These drugs did not inhibit significantly the liver SDH activity in normal rats. These results have been discussed in relation to their implications on intermediary metabolism.

THE EFFECTS of anti-inflammatory drugs on various aspects of intermediary metabolism have been investigated by several workers.[1–5] It has been shown that certain anti-inflammatory drugs probably uncouple oxidative phosphorylation by reaction with the key thiol (-SH) groups of certain enzymes connected with energy conservation.[6]

Glutathione (GSH) is one of the major thiols involved in the activity of a number of enzymes connected with intermediary metabolism. The fact that the synthesis of GSH is dependent upon the supply of ATP[7] raises an interesting question as to whether the anti-inflammatory drugs inhibit GSH synthesis during inflammation.

Inhibition of succinic oxidase, an important respiratory enzyme, by anti-inflammatory drugs has been reported.[8,9] However, there is no definite proof regarding the inhibition of succinic dehydrogenase (SDH) by anti-inflammatory drugs during inflammation.

The present investigation is designed to study the effects of some anti-inflammatory drugs on blood and liver GSH levels and SDH activity of liver during carrageenin induced edema and cotton pellet granuloma.

MATERIALS AND METHODS

Male albino rats (Haffkine strain) weighing 80–100 g were used. The anti-inflammatory drugs used in this study, hydrocortisone, phenylbutazone, and indomethacin, were administered orally as uniform suspensions in 1% (w/v) carboxymethyl cellulose (CMC).

Carrageenin edema test. Edema was produced acutely by injection of a phlogistic agent, carrageenin, into the plantar region of the hind paws of the rat according to the method of Winter *et al.*[10] Hydrocortisone (20 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 of body weight, followed by 4·0 ml of distilled water. The latter treatment seemed necessary to ensure uniform hydration of animals and to reduce the variability of edema formation. The control animals received 1·0 ml of the vehicle/100 g of body weight followed by 4·0 ml of distilled water. After exactly 1 hr, 0·1 ml of 1% (w/v) carrageenin was injected into the plantar region of each hind paw of the rats. The animals were anesthetized with ether 3 hr after injection of carrageenin, blood was collected by cardiac punctures, and liver was removed for biochemical studies.

Cotton pellet granuloma test. The cotton pellet granuloma was produced in rats by the method of Winter and Porter[11] with slight modification. 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 anesthesia. The drugs, hydrocortisone (20 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 of body weight daily for 7 days. Controls received 1·0 ml vehicle/100 g of body weight for the same period. On the eighth day the animals were sacrificed to collect blood and liver for biochemical studies.

Liver succinic dehydrogenase activity. A portion of the liver was homogenized in 0·1 M phosphate buffer (pH 7·4) to give a 10% (w/v) homogenate. SDH activity was determined by the tetrazolium method of Kun and Abood.[12]

Determination of glutathione. Glutathione levels of blood and liver were determined by the nitroprusside method of Grunert and Philips.[13]

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

RESULTS

Effect of anti-inflammatory drugs on blood and liver glutathione levels in carrageenin edema. There was no significant change in liver and blood GSH levels of normal and carrageenin-treated animals (Table 1). Pretreatment with hydrocortisone (20 mg/kg), phenylbutazone (100 mg/kg), and indomethacin (5 mg/kg) lowered significantly liver GSH levels in carrageenin-treated as well as in normal rats. However, blood GSH levels were insignificantly lowered by hydrocortisone and phenylbutazone in carrageenin-treated and normal rats; indomethacin pretreatment reduced blood GSH significantly (Table 1).

Effect of anti-inflammatory drugs on blood and liver glutathione levels in cotton pellet granuloma. There was no significant change in blood and liver GSH levels during cotton pellet granuloma (Table 2). Hydrocortisone, phenylbutazone, and indomethacin reduced liver and blood GSH to a lesser extent in normal rats and more markedly in

TABLE 1. EFFECT OF ANTI-INFLAMMATORY DRUGS ON BLOOD AND LIVER GLUTATHIONE (GSH) IN CARRAGEENIN EDEMA*

		Control	Hydrocortisone (20 mg/kg)	Phenylbutazone (100 mg/kg)	Indomethacin (5 mg/kg)
	No. of rats	10	6	6	6
Blood GSH (mg/100 ml)	Normal	34·4 ± 2·4	29·0 ± 3·1	29·3 ± 2·8	27·0 ± 2·6 (P < 0·02)
	Decrease with drug (%)		15·0	15	21·5
	Carrageenin edema	37·2 ± 3·0	30·0 ± 2·8	30·0 ± 1·4	28·4 ± 1·8 (P < 0·02)
	Decrease with drug (%)		11	16	24·0
Liver GSH (mg/100 g)	Normal	165·5 ± 2·9	140·0 ± 2·8	135·0 ± 3·4	138·0 ± 10·2
	Decrease with drug (%)		15 (P < 0·02)	18 (P < 0·01)	16 (P < 0·05)
	Carrageenin edema	165·8 ± 7·6	137·0 ± 1·8 (P < 0·01)	118·0 ± 4·4 (P < 0·001)	140·0 ± 6·5 (P < 0·05)
	Decrease with drug (%)		17·5	29	16

* Results are expressed as mean ± S.E.

TABLE 2. EFFECT OF ANTI-INFLAMMATORY DRUG ON BLOOD AND LIVER GLUTATHIONE IN COTTON PELLET GRANULOMA*

		Control	Hydrocortisone (20 mg/kg)	Phenylbutazone (100 mg/kg)	Indomethacin (5 mg/kg)
	No. of rats	10	8	8	8
Blood GSH (mg/100 ml)	Normal	35·0 ± 2·6	28·6 ± 1·8 (P < 0·05)	29·4 ± 2·4 (P < 0·05)	28·0 ± 3·4 (P < 0·04)
	Decrease with drug (%)		19	17	20
	Cotton pellet granuloma	41·1 ± 4·2	26·0 ± 3·2 (P < 0·02)	29·2 ± 1·9 (P < 0·02)	28·2 ± 1·95 (P < 0·02)
	Decrease with drug (%)		37	29	32
Liver GSH (mg/100g)	Normal	170·0 ± 3·9	142·0 ± 2·28 (P < 0·03)	128·0 ± 4·8 (P < 0·02)	130·0 ± 6·2 (P < 0·02)
	Decrease with drug (%)		16	25	24
	Cotton pellet granuloma	185·0 ± 6·9	132·0 ± 2·4 (P < 0·01)	104·0 ± 6·4 (P < 0·001)	128·0 ± 8·4 (P < 0·02)
	Decrease with drug (%)		28	44	31

* Results are expressed as mean ± S.E.

TABLE 3. EFFECT OF ANTI-INFLAMMATORY DRUGS ON LIVER SUCCINIC DEHYDROGENASE ACTIVITY DURING CARRAGEENIN EDEMA

No. of rats		Drug	Dose (mg/kg)	Liver succinic dehydrogenase units* (Mean ± S.E.)	Decrease with drug (%)
6	Carrageenin edema			880 ± 24·8	
7		Hydrocortisone	20	230 ± 14·2 ($P < 0.001$)	73·8
7		Phenylbutazone	100	377 ± 29·0 ($P < 0.001$)	57·2
7		Indomethacin	5	394 ± 27·4 ($P < 0.001$)	55·2
6	Normal			305 ± 19·8	
5		Hydrocortisone	20	264 ± 36·2	13·0
6		Phenylbutazone	100	259 ± 15·0	15·0
5		Indomethacin	5	283 ± 17·0	7·0

* The enzyme activity is expressed as micrograms of triphenyltetrazolium chloride reduced per gram of liver per 30 min at 37°.

TABLE 4. EFFECT OF ANTI-INFLAMMATORY DRUGS ON LIVER SUCCINIC DEHYDROGENASE ACTIVITY DURING COTTON PELLET GRANULOMA

No. of rats		Drug	Dose (mg/kg)	Liver succinic dehydrogenase units* (Mean ± S.E.)	Decrease with drugs (%)
10	Cotton pellet granuloma			928 ± 22·4	
8		Hydrocortisone	20	240 ± 16·4 ($P < 0.001$)	75
8		Phenylbutazone	100	360 ± 24·4 ($P < 0.001$)	61
8		Indomethacin	5	384 ± 27·4 ($P < 0.001$)	59
10	Normal			305 ± 19·8	
8		Hydrocortisone	20	248 ± 20·4 ($P > 0.05$)	19
8		Phenylbutazone	100	266 ± 20·9 ($P > 0.2$)	14
8		Indomethacin	5	274 ± 18·8 ($P > 0.3$)	11

* The enzyme activity is expressed as micrograms of triphenyltetrazolium chloride reduced per gram of liver per 30 min at 37°.

rats with cotton pellet granuloma. The inhibition in both groups (normal and cotton pellet) by these drugs is significant (Table 2).

Effect of anti-inflammatory drugs on liver succinic dehydrogenase activity during carrageenin edema. SDH activity in liver was increased markedly in carrageenin edema (Table 3). Pretreatment with hydrocortisone, phenylbutazone, and indomethacin inhibited significantly the increase of liver SDH activity induced by carrageenin edema. Liver SDH activity in normal rats was not significantly affected by any of these drugs.

Effect of anti-inflammatory drugs on liver succinic dehydrogenase activity during cotton pellet granuloma. Liver SDH activity was increased by 3-fold in cotton pellet granuloma when compared to normal rats (Table 4). Hydrocortisone, phenylbutazone, and indomethacin inhibited liver SDH activity significantly. The above drugs did not inhibit liver SDH activity significantly in normal rats.

DISCUSSION

The present study indicates that blood and liver GSH levels of rats are not significantly altered in carrageenin edema or cotton pellet granuloma (Tables 1 and 2). The three anti-inflammatory drugs tested, viz. hydrocortisone, phenylbutazone and indomethacin, lowered blood and liver GSH levels in normal rats as well as in rats subjected to both types of inflammation (Tables 1 and 2).

Our observations are supported by the recent evidence that liver GSH levels are not altered during carrageenin-induced pedal edema and that anti-inflammatory drugs such as phenylbutazone and indomethacin have no effect on liver GSH levels.[14]

Marozzi and Malone[15] indicated a possible correlation between a compound's ability to prevent hepatic GSH depletion in hind-leg tourniquets in rats and its anti-inflammatory potency against carrageenin-induced pedal inflammation. However, the findings in the present study show that such a correlation may not exist. In our study it was found that there is no relationship between the effects of drugs and their relative anti-inflammatory potencies. Our findings are also contrary to the report that serum sulfhydryl levels, which are decreased in rheumatoid arthritis, are then elevated by adrenocorticosteroid treatment.[16]

In this study we have observed a significant increase in liver SDH activity of rats in carrageenin edema as well as in cotton pellet granuloma. It is interesting to note that all the anti-inflammatory drugs tested significantly inhibited the liver SDH activity in both types of inflamation. There is no direct experimental evidence concerning the precise role of SDH in inflammation, although it has been shown that anti-inflammatory drugs such as salicylates and hydrocortisone are effective inhibitors of SDH activity.[17,18] SDH is one of the key enzymes linked with the energy (ATP) yielding citric acid cycle in living cells. The stimulation of SDH activity observed in both types of inflammation in this study would mean an increased supply of ATP to liver and possibly other tissues including the inflamed tissue.

The inhibition of liver SDH activity by anti-inflammatory drugs as seen in this study would result in the depletion of ATP supply to the liver tissue. This may be true in the the case of inflamed tissue as well, although there is no direct experimental evidence to support this assumption.

Similar studies in other types of inflammation such as adjuvant arthritis are in progress.

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