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BIOCHEMICAL STUDY OF THE ANTI-INFLAMMATORY ACTIVITY OF α AND β-AMYRIN ACETATE

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Abstract—The anti-inflammatory activity of α-amyrin acetate and β-amyrin acetate was found similar to hydrocortisone on granulation tissue formation induced by "cotton wool" pellet implantation in albino rats. Quantitatively, β-amyrin acetate showed more potent anti-inflammatory activity in comparison to α-amyrin acetate.

Hydrocortisone, α-amyrin acetate and β-amyrin acetate prevented the increased serum aspartate aminotransferase and serum alanine aminotransferase level during inflammation. These agents reduced serum alanine aminotransferase but not the serum aspartate aminotransferase level in normal animals. ATP phosphohydrolase activity in liver homogenates remained unchanged during inflammation but was significantly elevated by β-amyrin acetate and hydrocortisone. The significance of these biochemical studies is discussed in relation to their anti-inflammatory activity.

THE EXACT mechanism of action of anti-inflammatory agents is not known. Uncoupling of oxidative phosphorylation[1–4] and decrease of enzyme aminotransferase[5–7] and the inhibition of biosynthesis of sulphated mucopolysaccharides[8] by anti-inflammatory agents may be related to their anti-inflammatory activity. Some of the triterpenoids viz. glycyrrhetic acid, α-amyrin acetate and β-amyrin acetate have been shown to possess anti-inflammatory activity.[9–11] The present investigation was carried out to study the anti-inflammatory activity of α- and β-amyrin acetate on granulation tissue formation by cotton pellet implantation method.[12] Furthermore, the effects of these drugs on the serum aminotransferase and tissue adenosine triphosphate (ATP) phosphohydrolase activity were studied to elucidate the mechanism of their anti-inflammatory activity.

METHODS

Anti-inflammatory studies

Adult albino rats weighing 80–100 g were divided into groups of six animals each. The pellets of surgical cotton weighing 9·0 ± 1 mg were sterilized in a hot air oven for 2 hr and implanted in both the axillae and groin under ether anaesthesia according to the method of Meier *et al.*[12] One group served as a control and one group each was treated with intraperitoneal injection of hydrocortisone (Glaxo) and test drugs (α- and β-amyrin acetate)* daily for 6 consecutive days. The drugs were dissolved in propylene glycol. The pellets were dissected out on 7th day under light ether anaesthesia. They

* Obtained from Utilization Research Laboratory, National Botanic Garden, Lucknow.

were dried for 2 hr at 150° and weighed after cooling. The results were statistically analysed and per cent anti-inflammatory effect calculated according to the formula:

$$\text{Per cent anti-inflammatory effect} = \left(1 - \frac{G_t}{G_c}\right) \times 100$$

where G_t and G_c are the weight of granulation tissues in treated and control groups respectively.

Biochemical studies

The enzyme estimations were done in normal albino rats and in rats subjected to formaldehyde induced arthritis according to the technique of Brownlee.[13] The animals weighing 100–110 g were divided into groups of six animals each. The arthritis was produced by injecting 0·1 ml of 2% (v/v) formaldehyde subcutaneously under the plantar aponeurosis. One group served as control and one group each was treated with intraperitoneal injection of hydrocortisone (1 mg/100 g) and test drugs (4 mg/100 g each) daily for 10 days. Serum was obtained from the blood collected after decapitation of the rats. The liver tissues were obtained immediately and pooled.

Serum L-asparatate: 2-oxoglutarate aminotransferase (EC 2.6.1.1, aspartate aminotransferase) and serum L-alanine: 2-oxoglutarate aminotransferase (EC 2.6.1.2, alanine aminotransferase) were estimated by the method of Reitman and Frankel.[14] One unit of enzyme activity was the change in the extinction (E) of 0·001/min/ml of serum. Extinction was measured by a Bausch and Lomb Spectronic '20' Colorimeter at 505 mμ.

ATP phosphohydrolase (EC 3.6.1.4) activity was assayed in 10% (w/v) homogenates of pooled liver tissue prepared in 0·25 M sucrose by Potter–Elvehjem homogenizer. The reaction mixture consisted of 0·05 M tris pH 8·0, 1 mM ATP and 0·1 ml of 10% tissue homogenates in a final volume of 2 ml. Release of P_i (inorganic phosphorus) from ATP was measured according to Fiske and SubbaRow.[15] The split of 1 μmole of P_i/100 mg of tissue in 15 min at 37° was considered as one unit of the enzyme activity. No ions were added from outside.

RESULTS AND DISCUSSION

Effect on granulation tissue formation by cotton pellet implantation

The effects of hydrocortisone (1 mg/100 g i.p.), α-amyrin acetate and β-amyrin acetate (4 mg/100 g i.p. each) were studied on granulation tissue formation by cotton pellet implantation method in albino rats. The results are shown in Table 1. α-Amyrin acetate and β-amyrin acetate showed significant anti-inflammatory activities ($P < 0·05$ and $< 0·001$ respectively) similar to hydrocrotisone ($P < 0·001$). β-Amyrin acetate was found to be more potent anti-inflammatory agent as compared to α-amyrin acetate. β-Amyrin acetate showed 43·6 and α-amyrin acetate 19·1 per cent anti-inflammatory effect when tested in equal doses.

The effect on serum aspartate aminotransferase and serum alanine aminotransferase

The effects of hydrocortisone, α-amyrin acetate and β-amyrin acetate on serum aminotransferases in normal and arthritic rats are shown in Tables 2 and 3. Both aspartate and alanine aminotransferases activities were significantly increased in the

TABLE 1. EFFECT OF HYDROCORTISONE, α AND β-AMYRIN ACETATE ON GRANULATION TISSUE FORMATION BY COTTON PELLET IMPLANTATION IN RATS

Compounds	Dose mg/100 g i.p.	Mean average weight of granulation tissue after 6 days (mg ± S.E.)	Anti-inflammatory effect (%)	P
Normal saline (Control)	0·5 ml	16·3 ± 0·42	—	—
Hydrocortisone	1·0	11·1 ± 0·23	31·3	<0·001
α-Amyrin acetate	4·0	13·2 ± 0·82	19·1	<0·05
β-Amyrin acetate	4·0	9·2 ± 0·21	43·6	<0·001

TABLE 2. EFFECT OF HYDROCORTISONE α AND β-AMYRIN ACETATE ON SERUM ASPARTATE AND ALANINE AMINOTRANSFERASES IN NORMAL ALBINO RATS

Drugs	Aspartate amino-transferase*	P value	Alanine amino-transferase*	P value
Normal saline (Control)	28·2 ± 1·2	—	32·1 ± 1·5	—
Hydrocortisone	27·8 ± 1·0	0·9 — 0·8	24·6 ± 1·2	<0·001
α-Amyrin acetate	28·0 ± 1·2	0·9 — 0·8	22·0 ± 1·3	<0·001
β-Amyrin acetate	26·4 ± 1·4	0·3 — 0·2	21·4 ± 1·1	<0·001

* Enzyme activity in units. One unit = change in extinction (E) 0·001/min/ml of serum.

TABLE 3. EFFECT OF HYDROCORTISONE α AND β-AMYRIN ACETATE ON SERUM ASPARTATE AND ALANINE AMINOTRANSFERASES IN ARTHRITIC ALBINO RATS

Drug	Aspartate amino-transferase*	P† value	Decrease by drug (%)	Alanine amino-transferase*	P† value	Decrease by drug (%)
Normal saline (Control)	28·2 ± 1·2	—	—	32·1 ± 1·5	—	—
Arthritic	50·4 ± 1·2	—	—	39·2 ± 1·5	—	—
Arthritic + Hydrocortisone	28·3 ± 1·3	<0·001	43·8	24·5 ± 1·1	<0·001	37·5
Arthritic + α-Amyrin acetate	42·8 ± 1·0	<0·05	13·0	27·4 ± 1·4	<0·001	30·1
Arthritic + β-Amyrin acetate	28·8 ± 1·1	<0·001	42·6	25·6 ± 1·2	<0·001	35·4

* Enzyme activity in units. One unit = change in the extinction (E) 0·001/min/ml of serum.
† Compared with the enzyme activity in arthritic animals.

serum during inflammation. α-Amyrin acetate and β-amyrin acetate prevented the increase in the enzyme activities due to the inflammatory reaction similar to that obtained by hydrocortisone. β-Amyrin acetate treatment showed a 42·6 per cent decrease in aspartate aminotransferase activity as compared to 13 per cent lowering by α-amyrin acetate. On the contrary, these drugs significantly decreased only the normal serum alanine aminotransferase activity but failed to alter the normal serum aspartate aminotransferase activity (Table 2). The reduction in the aminotransferase activities by these agents may be related to their anti-inflammatory activity or may be an independent action on the enzyme. Since both α- and β-amyrin acetate like hydrocortisone did not effect the serum aspartate aminotransferase activity in the normal animals and significantly reduced the enzymatic activity in the arthritic rats, it may be suggested that the anti-inflammatory activity of these agents was related to their action on the enzyme serum aspartate aminotransferase. Furthermore, quantitative relationship between anti-inflammatory and serum aspartate aminotransferase activity was also observed with α- and β-amyrin acetate. However, no correlation of anti-inflammatory activity of these drugs could be made with the inhibition of alanine aminotransferase activity since this enzyme activity was inhibited by these drugs in normal animals as well. Similar effects on the serum alanine and aspartate aminotransferases were also observed with salicylates,[6,16] salicylic acid congeners,[17] glycyrrhetic acid[18] and imipramine.[19]

Effect on ATP phosphohydrolase activity in pooled liver tissue homogenates

The effects of α- and β-amyrin acetate on ATP phosphohydrolase activity in pooled liver homogenates obtained from normal and arthritic rats are shown in Table 4. The present study showed that inflammatory reaction did not alter this enzyme activity in liver homogenates. β-Amyrin acetate, a potent anti-inflammatory agent, significantly increased the ATP-phosphohydrolase activity in liver homogenates both in normal and arthritic animals. Similar finding was also obtained with hydrocortisone. However, α-amyrin acetate which showed weak anti-inflammatory activity failed to alter liver ATP-phosphohydrolase activity.

Whitehouse and Haslam[20] observed that several anti-inflammatory agents uncoupled oxidative phosphorylation and increased ATP-phosphohydrolase activity in liver mitochondria. Falcone[21] reported that salicylates cause uncoupling of oxidative

TABLE 4. EFFECT OF HYDROCORTISONE α AND β-AMYRIN ACETATE ON THE ATP PHOSPHOHYDROLASE ACTIVITY IN THE POOLED LIVER HOMOGENATES OBTAINED FROM NORMAL AND ARTHRITIC RATS

		Control	Hydro-cortisone	α-Amyrin acetate	β-Amyrin acetate
Liver ATP Phospho-hydrolase*	Normal	10·70	16·10	10·70	11·68
	Increase with drug (%)	—	50·4	—	9·1
	Arthritic	10·73	16·09	10·73	11·62
	Increase with drug (%)	—	49·0	—	8·0

* Expressed in μmoles of P_i Split for 100 mg of tissue in 15 min at 37°.

phosphorylation by stimulating the ATP-phosphohydrolase activity. However, in our study the enzyme ATP-phosphohydrolase activity was unaffected by the inflammatory reaction, therefore the increased ATP-phosphohydrolase activity by hydrocortisone and β-amyrin acetate may not be related to their anti-inflammatory activity.

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