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The effect of anti-inflammatory agents on the changes in local lymph after thermal injury

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Summary

1. Lymph was collected directly from the hind limbs of rabbits anaesthetized with pentobarbitone and from unanaesthetized rabbits before and after one hind limb was injured by immersion in water at 60° C for 1 minute.

2. Rabbits were treated with anti-inflammatory agents hydrocortisone or indomethacin which, in acute experiments, were infused close-arterially into the limb either at the time of the injury or 90 min later. In chronic experiments hydrocortisone was given intravenously three times a day.

3. When given at the time of the injury both drugs reduced the subsequent mean increases in the lymph of the intracellular enzymes lactate dehydrogenase and glutamic pyruvate transaminase but not those of β-glucuronidase and protein; whereas when given 90 min after the injury only the increase in lymph protein concentration was reduced.

4. The results indicate that these anti-inflammatory agents probably inhibit the second phase of increase in vascular permeability which occurs after injury and in addition, reduce the leakage of intracellular protein by a non-specific effect on membrane permeability.

5. The pronounced variability of the response of individual animals and the complexity of the experiments preclude the method as a suitable model for the estimation of anti-inflammatory activity.

Introduction

In earlier investigations it was shown (Lewis, 1967, 1969) that thermal injury to one hind limb resulted in the appearance of certain intracellular enzymes in the lymph draining the limb. The study of different types of injury revealed that the nature and pattern of enzymes in the lymph draining the injured area reflected to a large extent the degree of cellular injury.

In the present experiments the effect of anti-inflammatory agents on the changes in the composition of the lymph after thermal injury has been investigated. The experiments were designed to examine the effect of a steroid, hydrocortisone, and a non-steroid, indomethacin when given at the time of the injury before the biochemical changes had occurred, as well as at some time after the injury when the changes in the composition of the lymph had almost reached their maximum. Finally the effect of hydrocortisone was studied when administered daily to an animal having an implanted lymphatic cannula from which hind limb lymph could be collected over several days.

Methods

Lymph collection

All experiments were performed on male New Zealand white rabbits weighing 2·5 to 3·3 kg, anaesthetized with pentobarbitone sodium. The dose of anaesthetic was usually about 40 mg/kg but was adjusted according to the depth of respiration and corneal and other reflexes.

The main femoral lymphatic was dissected out and cannulated with a polythene cannula. In acute experiments the limb was moved passively to facilitate the collection of lymph, as described previously (Lewis & Westcott, 1968). In chronic experiments the lymphatic was cannulated as described by Lewis (1969). In all experiments one hind limb was subjected to thermal injury by immersion in water at 60–62° C for 1 minute.

Drug administration

In acute experiments the drugs used were administered by retrograde infusion to the femoral artery by close intra-arterial infusion to the inferior epigastric artery. Rabbits were given 100 mg hydrocortisone sodium succinate or 50 mg indomethacin, either immediately after or 90 min after the injury. Each infusion continued for 30 minutes.

In the chronic experiments the animals were given each day, hydrocortisone sodium succinate (10 mg/kg) in 3 doses at 8 hourly intervals commencing immediately after the injury. Injections were made into the ear vein.

Biochemical methods

Description of the analysis and calculation of units of the following three enzymes were given previously by Lewis (1969): lactate dehydrogenase (LDH) (L-lactate: NAD oxidoreductase, E.C.1.1.1.27); glutamic pyruvate transaminase (GPT) (L-alanine: 2: oxoglutarate aminotransferase, 2.6.1.2); β-glucuronidase (β-gluc) (β-D-glucuronide glucuronohydrolase, E.C.3.2.1.31).

Protein concentration was determined by the method of Lowry, Roseborough, Farr & Randall (1951).

Drugs used

Hydrocortisone sodium succinate was prepared from the intermediate, hydrocortisone 21-hemi-succinate (Upjohn), by titration with N/10 NaOH to neutrality using phenolphthalein as indicator. Indomethacin capsules (Merck, Sharpe & Dohme) containing 25 mg were extracted into alcohol and diluted with McEwen's solution.

Results

Acute experiments

Lymph flow: The increase in lymph flow following thermal injury to the hind limb is shown in Table 1 for the treated and untreated animals. In none of the drug-treated animals was it possible to detect any significant difference from the control animals. However, in those receiving 100 mg hydrocortisone infused

immediately after the burn, lymph flow did not reach a maximum for several hours while in the untreated animals lymph flow was maximal at 1 h and subsequently declined.

Protein: After one hind limb of untreated rabbits was burned at 60° C for 1 min there was a rapid increase in the lymph protein concentration as shown in Table 1. The results in Table 1 also show that there was no difference in the mean concentration between untreated and treated animals when the drugs were given at the time of injury. This suggests that the leakage of plasma protein into the lymph was unaffected. However, after the thermal injury in the untreated rabbits, the increase in protein concentration in lymph continued throughout the duration of the experiment (i.e. 4–6 hours). This was not so in animals in which the anti-inflammatory drugs were administered 90 min after the injury. In these experiments both hydrocortisone and indomethacin reduced the increase of the

TABLE 1. *Concentration of intracellular enzymes, and protein in lymph and flow of lymph collected before and after burning at 60° C for 1 min, from untreated rabbits and from rabbits treated with hydrocortisone or indomethacin*

Assay	Untreated	100 mg Hydrocortisone	50 mg Indomethacin
Protein (mg/ml)			
Control	22±2	22±2	28±2
1	36±3	34±3	41±3
2	36±3	35±4	38±3
3	32±3	36±5	38±4
4	33±2	36±4	41±3
5	36±2	38±4	43±3
LDH (mu/ml)			
Control	445± 75	733± 114	505± 86
1	4,884±1,740	4,245±1,835	1,825± 563†
2	8,223±3,250	3,508±1,487	3,614±1,153†
3	11,381±4,034	4,399±1,936	4,351±1,137
4	11,664±3,028	3,121± 933*	6,782±2,139
5	9,331±2,612	3,823±1,161†	8,323±2,455
GPT (u/l.)			
Control	6±1	9±1	8±2
1	34±8	16±2†	21±4
2	39±8	17±3*	28±6
3	34±6	15±3*	30±6
4	43±8	22±3*	31±5
5	41±8	23±5†	33±7
β-Gluc. (u/100 ml)			
Control	295± 85	278± 67	295± 66
1	781±189	665±237	729± 64
2	619±106	897±240	1,065±269
3	720±176	1,073±276	863±196
4	772±182	1,276±235	790±137
5	752±164	1,051±246	881±237
Lymph flow (μl/min)			
Control	11·4±2·4	10·5±1·9	11·7± 1·8
1	43·1±6·2	29·2±6·2	38·3±11·1
2	38·9±4·2	29·2±3·5	33·3± 7·2
3	31·4±3·1	32·8±3·4	27·7± 3·7
4	25·7±3·5	33·3±5·7	26·7± 3·7
5	26·4±4·0	32·8±5·8	26·7± 3·7

The values are the means of 6 experiments ± S.E. The numerals in the assay column represent hours after injury and the start of the infusion. All values obtained from treated rabbits were compared with the respective value in the untreated rabbits to determine the P value. * Indicates $P<0{\cdot}05$; † indicates $P=0{\cdot}05$.

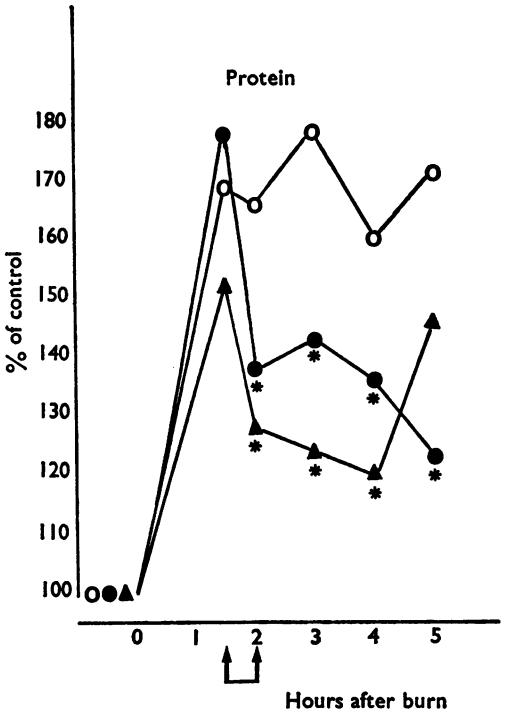

FIG. 1. Protein concentration of hind limb lymph collected before and after the hind limb was injured at 60° C for 1 min (at 0 time), from untreated rabbits (○——○) and from rabbits receiving a 30 min intra-arterial infusion (indicated by the arrows) of 100 mg hydrocortisone (●——●) or 50 mg indomethacin (▲——▲). The results are the means of 6 experiments and the points of significant difference ($P<0{\cdot}05$) are indicated by asterisks.

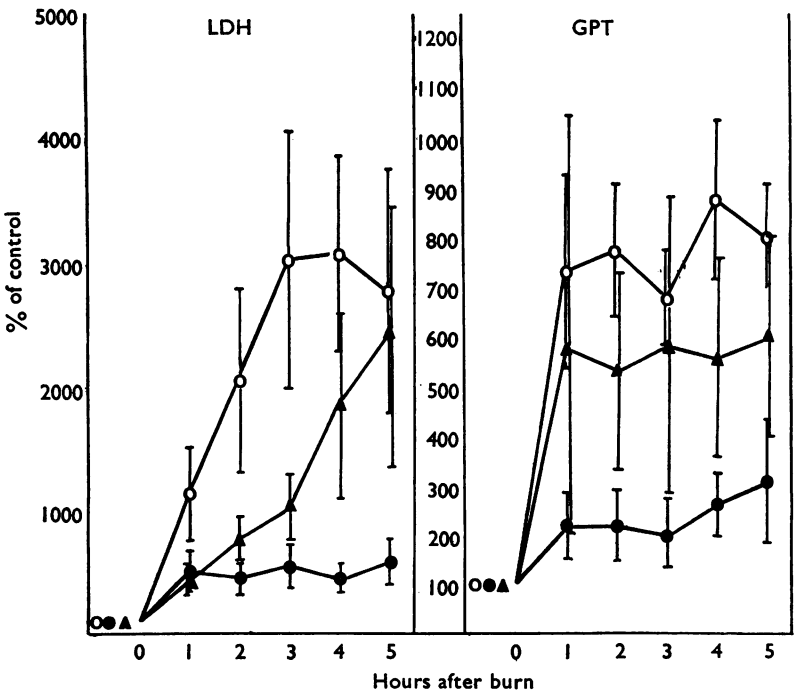

FIG. 2. Concentration of LDH and GPT in hind limb lymph collected before and after the hind limb was injured at 60° C for 1 min (at 0 time), from untreated rabbits (○——○), and from rabbits receiving 100 mg hydrocortisone (●——●) or 50 mg indomethacin (▲——▲) given in a 30 min intra-arterial infusion commencing immediately after the injury. The results are means of 6 experiments ± S.E.

protein concentration in the lymph, as illustrated in Fig. 1. The times at which the difference of the means, before and after the infusion, were statistically significant are also indicated in Fig. 1.

LDH and GPT: The mean increase in LDH and GPT activities in lymph following injury was lower in treated, than in untreated animals when the drugs were given at the time of the injury as shown in Fig. 2. The results in Table 1 show the points of significance between the two groups. However, no difference in the increase of activities of either enzyme could be shown when either anti-inflammatory agent was given 90 min after the injury.

β-Glucuronidase: When the anti-inflammatory agents were given either at the time of the injury (Table 1), or 90 min later there was no reduction in the increase in β-glucuronidase levels in the lymph.

Chronic experiments

In six experiments in rabbits having an indwelling lymphatic cannula the effect of daily administration of hydrocortisone (10 mg/kg) was examined. As in the acute experiments the mean increase in lymph levels of LDH and GPT after injury was less in the treated than in the untreated animals. However, there was

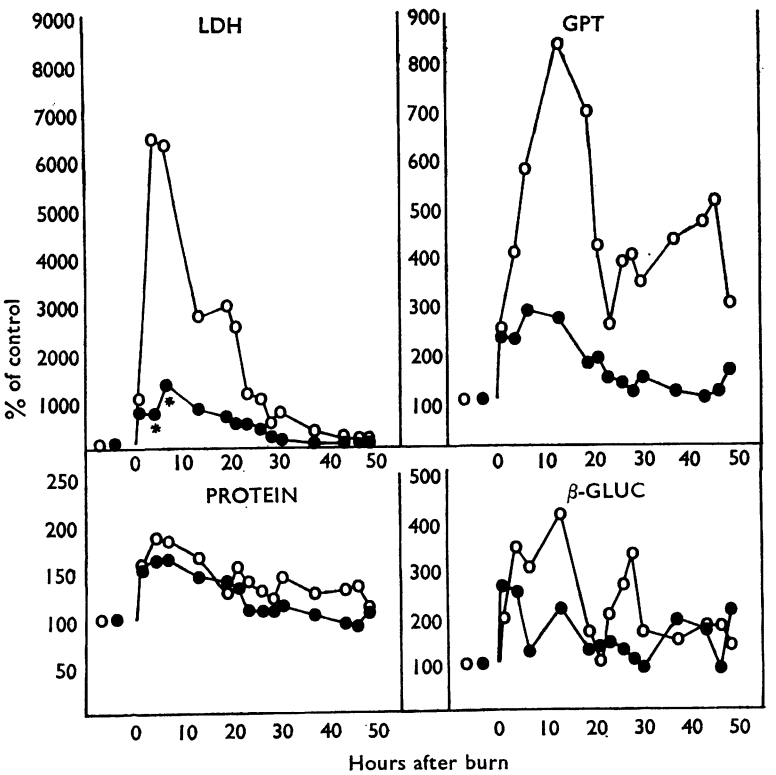

FIG. 3. Concentration of LDH, GPT, protein and β-glucuronidase in hind limb lymph collected, before and after the hind limb was injured at 60° C for 1 min (at 0 time), from untreated rabbits (○——○) and from rabbits treated with hydrocortisone (total dose, (10 mg/kg)/day) injected intravenously 3 times a day (●——●). The results are means of 3 experiments and the points of significant difference ($P<0.05$) indicated by an asterisk.

a statistically significant difference at only two time intervals after injury as indicated in Fig. 3. The results illustrated in Fig. 3 also show that there was no significant effect on the increased lymph protein concentration and only a small insignificant effect on the increased concentration of β-glucuronidase.

Histology

Histological examination of the skin taken 72 h after thermal injury showed that a large proportion of the epithelium had disappeared and much of that which remained contained pyknotic nuclei. In addition there was evidence of pronounced oedema, vasodilatation, vascular stasis and infiltration of polymorphonuclear leucocytes. Although these signs were generally less pronounced in the three rabbits treated with hydrocortisone, in only one of these did the histological changes appear to be prevented. In this animal much of the epithelium remained and the migration of leucocytes and vascular stasis were less marked.

Discussion

Although the appearance of intracellular enzymes in the lymph draining an injured tissue is an indicator of the degree of cellular injury, the method does not appear to be suitable for use as a model for the measurement of anti-inflammatory activity because of a large degree of variability. The use of a large number of experiments to overcome this variability is not practicable since the experiments themselves are complex and time consuming. The variability can be readily seen from the results of Figs. 2 & 3 where although the anti-inflammatory agents reduced the mean levels of lymph enzymes considerably there were few points of significant difference since there were large standard errors in the control experiments.

However, despite the variability inherent in this method some statistically significant differences were obtained. One point of interest was the effect which the anti-inflammatory agents had on the lymph protein concentration. Although no effect was observed when the drugs were infused immediately after the injury, when they were infused 90 min after burning, the lymph protein was significantly reduced. Since Lewis & Wawretschek (1971) using the same technique showed that there were two phases of increase in lymph protein it is possible that it is this second phase, which corresponds to a second phase of increased vascular permeability (Sevitt, 1958 ; Burke & Miles, 1958) which was inhibited by the anti-inflammatory agents. The fact that neither hydrocortisone nor indomethacin affected the increase in lymph β-glucuronidase activity is consistent with this view, since it has been shown (Lewis, 1969) that β-glucuronidase leaks into the lymph after injury not from the injured cells but from the plasma as a result of the initial increase in vascular permeability.

On the other hand it is possible that the anti-inflammatory agents inhibit the delayed leakage of intracellular protein into the lymph which has been observed after thermal injury (Winsey, 1972). Both drugs, in the high dose used, reduced the mean increase of the intracellular enzymes LDH and GPT in acute experiments and hydrocortisone had a similar effect when a 'therapeutic' dose level was administered during chronic experiments.

However, neither drug had any effect when given to the animal at a time when the changes in lymph due to the injury had already reached their maximum. This finding suggests that these anti-inflammatory agents might act primarily by inhibiting acute tissue changes. Such a suggestion is not inconsistent with their therapeutic usefulness in chronic inflammation since the recurrence of acute tissue changes must be part of the chronic condition.

In such a small series of experiments it was not possible to establish more than a rough correlation between biochemical changes and histological appearance. Lysosomal enzymes do not generally appear in the lymph after a thermal injury and it seems unlikely that they play a role in the early phase of this type of tissue injury. But although these experiments do not add anything to the current theories of anti-inflammatory agents stabilizing lysosomal membranes (Weissman & Thomas, 1964), they might indicate a more general and less specific action on overall membrane stabilization.

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