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ANTI-INFLAMMATORY AND IRRITANT EFFECTS OF A FRACTION FROM NORMAL HUMAN PLASMA

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1 By the use of carrageenan-induced rat paw oedema assay the anti-inflammatory activity of a fraction isolated from normal human plasma has been measured after its intravenous, intraperitoneal and oral administration. Its effects in the dextran-induced rat paw oedema and the systemic dextran anaphylactoid reaction in the rat were also studied.

2 The fraction showed marked anti-inflammatory activity in the carrageenan test after intravenous administration and a smaller but still significant activity when given intraperitoneally, but was inactive orally after the administration of a larger dose. It was active in the dextran-induced paw oedema test but not against the anaphylactoid reaction.

3 A comparison between its anti-inflammatory and irritant properties revealed no correlation when each parameter was determined in relation to dose. The fraction did not affect the blood pressure of the anaesthetized rat.

4 These findings are discussed in relation to the existence of a natural anti-inflammatory substance or substances in human plasma.

Introduction

A fraction showing anti-inflammatory activity in the carrageenan-induced rat paw oedema test has been isolated from normal human serum (McArthur, Smith & Freeman, 1972). A much simpler preparation of this material from pooled human plasma was subsequently described (Ford-Hutchinson, Insley, Elliott, Sturgess & Smith, 1973). The substance or substances responsible for the experimental anti-inflammatory properties are resistant to acid and proteolytic digestion, are insoluble in organic solvents and have an apparent molecular weight of 1,000 or less.

In the present study the effects of varying the route of administration of the plasma fraction on its anti-inflammatory activity in the carrageenan test have been investigated. In addition, the possibilities that this activity could be explained either by a counter-irritant phenomenon or by an alteration in blood pressure were examined. The activity of the plasma fraction on other experimental tests for anti-inflammatory properties has also been studied.

Methods

Female albino Wistar rats (Oxfordshire Laboratory Animal Colonies, Southern Ltd; 150-200 g) were used.

Preparation of plasma fraction

The procedure used was that described by Ford-Hutchinson *et al.* (1973). The plasma fraction used in the present study was their combined Fractions II and IV, prepared from pooled human plasma (Blood Transfusion Centre, Tooting, London, S.W.17) by ultra-filtration in an Amicon 2L cell under an atmosphere of N_2 with a Diaflo PM 10 membrane. The concentrated ultrafiltrate was applied to a Sephadex G 25 fine column and then eluted with distilled water.

Assay of anti-inflammatory activity

Carrageenan-induced rat paw oedema. The method used was that of Winter, Risley & Nuss (1962). In the control group each animal received either 1 ml 0.9% w/v NaCl solution (saline) by injection or 3 ml saline orally and in the experimental groups each rat was either injected with 1 ml of the plasma fraction or received 3 ml orally. All the injections in these and the subsequent experiments were filtered through Millipore Millex filter units (type GS 0.22 μm pore size) before administration either into a tail vein or intraperitoneally. The saline and plasma fractions were given either orally or by injection 30 min before

the injection of 0.1 ml 1.0 g/100 ml (w/v) carrageenan (Viscarin Marine Colloids) in saline in the plantar region of the right hind foot. Foot volumes were measured with a mercury plethysmograph (Arnold R. Horwell, Ltd, London) immediately after the injection of the carrageenan (0 h) and at hourly intervals for 4 hours. The results were calculated as mean percentage increases in the volume of the injected paw compared to the value at 0 hours.

Dextran-induced rat paw oedema. A similar procedure to the carrageenan test was used except that the subplantar injection consisted of 0.1 ml 1 g/100 ml (w/v) clinical dextran, average molecular weight 110,000 (Fisons Ltd) and the foot volumes were measured at intervals of 15 min, 30 min, 1 h, 2 h and 3 hours.

Systemic dextran anaphylactoid reaction. The test was carried out according to the method of Voorhees, Baker & Pulaski (1951), each animal being given an intraperitoneal injection of 185 mg/kg of clinical dextran at 0 hours. Each control rat received 1 ml of saline and each experimental animal was given 1 ml of plasma extract by intravenous injection 30 min before the dextran. The severity of the oedema and erythema which developed in each paw, ear and the nose was assessed on an arbitrary scale (0-3 units, giving a maximum possible score of 21 for each animal) at intervals of 30 min, 45 min, 1 h, 2 h and 3 hours.

Comparison of irritant and anti-inflammatory activities of the plasma fraction. Several preparations of the plasma fraction, equivalent to 4 litres of original plasma ultrafiltrate, were bulked, reduced to dryness in a rotary evaporator at 37°C under reduced pressure and the residue dissolved in 20 ml saline (bulk preparation solution). Graded dilutions of this solution were prepared with saline, and assayed for anti-inflammatory activity by the carrageenan test and for irritancy by the procedure described by Atkinson, Boura & Hicks (1969). This technique involves the subplantar injection of 0.1 ml of either the plasma fraction preparation or saline and measurement of the increase in foot volume at hourly intervals for 4 hours.

Effect of plasma fraction on the blood pressure of the anaesthetized rat

The blood pressure of rats, anaesthetized by the intraperitoneal injection either of urethane (1.25 g/kg body wt) or of pentobarbitone sodium (45 mg/kg body wt), was monitored after catheterization of the carotid artery by a Devices Recorder equipped with a Bell and Howell Physiological Pressure transducer. After a stable blood pressure tracing had been obtained each animal received 1 ml of plasma fraction intravenously and the blood pressure was monitored for 1-2 hours. This was followed by the intravenous injection of 1 ml saline, acetylcholine, 100 μg in 0.1 ml saline and finally noradrenaline, 3 μg in 0.3 ml saline. Further experiments were performed in which repeated doses of the plasma fraction were injected at intervals over a period of 2 hours.

Results

Table 1 shows that the intravenous injection of 1 ml of the plasma fraction caused a significant reduction in the carrageenan-induced paw oedema over a period of 2-4 h after the administration of the irritant. A smaller and less prolonged anti-inflammatory effect was produced by the intraperitoneal injection of an equal amount of the

Table 1 Inhibitory effects of plasma fraction on carrageenan-induced rat paw oedema after intravenous, intraperitoneal and oral administration.

Time (h) after carrageenan injection	*Route of administration*					
	Intravenous		*Intraperitoneal*		*Oral*	
	Control (1)	*Expt. (1)*	*Control (1)*	*Expt. (1)*	*Control (3)*	*Expt. (3)*
1	22.9 ± 7.7	20.7 ± 5.7	22.5 ± 7.4	19.8 ± 7.4	22.5 ± 7.3	23.6 ± 5.5
2	75.2 ± 10.1	32.1 ± 12.4*	83.1 ± 11.5	57.2 ± 11.5*	76.9 ± 22.1	73.4 ± 27.6
3	93.8 ± 12.3	48.0 ± 8.8*	97.4 ± 9.0	73.3 ± 15.4*	94.6 ± 15.6	87.0 ± 27.4
4	93.1 ± 12.3	66.6 ± 9.1*	94.9 ± 9.3	83.6 ± 16.5	98.4 ± 14.8	93.8 ± 23.6

The results, expressed as percentage increase in paw volume relative to 0 h, are given as mean ± s.d. Those marked * show a statistically significant difference ($P < 0.001$) from the corresponding control animals. Each group consisted of 10 rats, the figures in brackets are the volume (ml) either of saline given to each control or of plasma fraction given to each experimental animal.

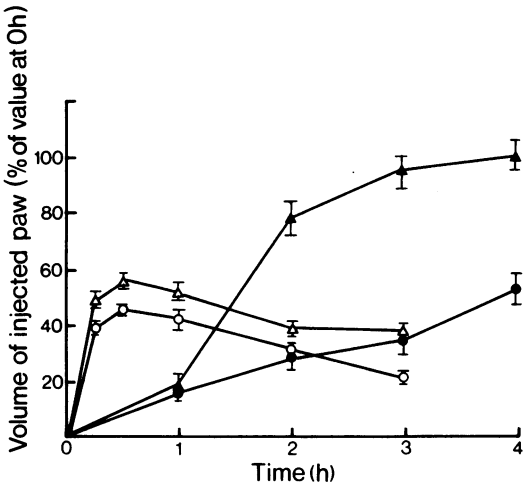

Fig. 1 Anti-inflammatory effects of plasma fraction in carrageenan- and dextran-induced rat paw oedema tests. Results calculated as volume of paw as a percentage of corresponding value at 0 h, given as means ±s.e., each experimental point representing 10 animals, for the following groups: (△) saline control, dextran test; (○) plasma fraction, dextran test; (▲) saline control, carrageenan test; (●) plasma fraction, carrageenan test.

plasma fraction. Preliminary experiments showed that 1 ml of the plasma fraction given orally was ineffective and Table 1 shows that a similar result was observed when the dose was increased to 3 ml.

The anti-inflammatory activities of the plasma fraction in the carrageenan and in the dextran rat paw oedema test are compared in Figure 1. The local dextran reaction was much less affected than the carrageenan test although a significant inhibition of paw swelling occurred in both. Table 2 shows that the systemic dextran anaphylactoid reaction was not affected by the plasma fraction.

The results of preliminary experiments showed that the plasma fraction possesses some local irritant activity when this was assessed by measuring the increase in paw volume following subplantar injection in the rat. The possible correlation of the anti-inflammatory activity of the carrageenan reaction and the irritant activity was investigated in the same bulk preparation solution by a comparison of dose-effect relationships for each activity. The irritant activity was assessed by the measurement of paw oedema at 4 h after the administration of the same dilution of the bulk preparation solution (see methods section) as was given 30 min before the administration of carrageenan in the anti-inflammatory test. The inhibition of paw oedema was calculated as the percentage reduction of the mean paw oedema at 3 h in the group given the plasma fraction compared to the group given saline. The results are illustrated in Figure 2. Over the time period investigated there was no correlation between the irritancy and the anti-inflammatory activity (correlation coefficient 0.64; $P > 0.4$). At several of the higher dilutions of the bulk preparation solution no significant irritant effect was observed whereas a statistically significant ($P < 0.05$) anti-inflammatory activity was found.

In the blood pressure experiments a single intravenous injection of 1 ml of the plasma fraction produced minor fluctuations within the range of those observed after a similar injection of 1 ml saline. In the same animal preparation the expected results were obtained after the administration of acetylcholine or noradrenaline. The repeated injection (4 x 1 ml injections over a 2 h period) of the plasma fraction produced neither acute nor cumulative effects on the blood pressure of rats anaesthetized with either urethane or pentobarbitone.

Discussion

The results of the present experiments show that a fraction, isolated from normal human plasma by ultrafiltration and column chromatography, possesses experimental anti-inflammatory properties. It is active in the carrageenan assay, less so in the dextran paw oedema test and inactive against the systemic dextran anaphylactoid reaction. The plasma fraction is most active in the carrageenan assay when given intravenously, is less effective by intraperitoneal injection and is not active when given in larger doses by the oral route. It is known

Table 2 Effect of plasma fraction on the systemic dextran anaphylactoid reaction in the rat.

Time after dextran	*Control (saline, 1 ml)*	*Experimental (plasma fraction, 1 ml)*
30 min	8 ± 2	9 ± 2
45 min	11 ± 2	11 ± 1
1 h	12 ± 2	12 ± 1
2 h	16 ± 3	16 ± 3
3 h	19 ± 2	18 ± 3

The results, expressed as the total scores, are given as means ± s.d. Each group consisted of 10 rats and there was no statistically significant difference between the values from the controls and experimental animals. The sample of plasma fraction used in the experimental group inhibited the carrageenan paw oedema reaction (10 rats) by 43% at 3 hours.

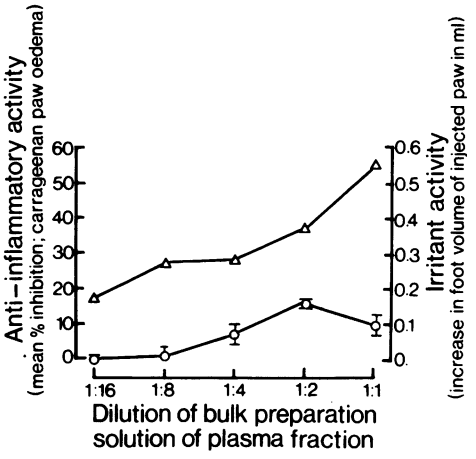

Fig. 2 Dose-response relationship of anti-inflammatory and irritant properties of plasma fraction (bulk preparation solution). The results in the anti-inflammatory (carrageenan) assay (△) are given as the percentage reduction of the mean paw oedema at 3 h in the group given the plasma fraction compared to the group given saline. Each experimental point represents 10 animals (five saline control, five plasma fraction) and a statistically significant difference ($P < 0.05$) between the two groups was found with each dilution of the bulk preparation. The results in the irritancy test (○) are given as increase in foot volume of the injected paw at 4 h expressed as means ±s.e., each experimental point representing five animals. The values were compared with those obtained from corresponding control groups (each of five rats) injected with an equivalent quantity of saline, and no statistically significant differences were found with the groups which received the 1 : 8 and 1 : 16 dilutions of the bulk preparation solution. The mean change (±s.e.) in foot volume (ml) between 0 and 4 h following the injection of 0.1 ml saline in the controls (25 animals) was −0.03 ± 0.02.

from other work (Ford-Hutchinson *et al.*, 1973) that the anti-inflammatory activity in the plasma fraction is stable both to acid digestion and to proteolytic enzymes. Its lack of effect after oral administration cannot therefore be due to its destruction by either acids or gastrointestinal enzymes but may be due to a failure of absorption from the gut.

Its decreased activity after intraperitoneal administration is against the suggestion that its anti-inflammatory activity may be due to a general irritant action (Büch & Wagner-Jauregg, 1962). Additional evidence for this view is that the dextran paw oedema test is more sensitive to intraperitoneal irritants, such as hypertonic saline and acetic acid, than is the carrageenan reaction (Garattini, Jori, Bernardi, Carrara, Paglialunga & Segre, 1965) whereas in the present work the carrageenan reaction is more sensitive to the plasma fraction. Atkinson & Hicks (1971) reported that the anti-inflammatory and local irritant activities of a sponge exudate showed a strong correlation and that this finding cast a doubt on the existence of a specific anti-inflammatory factor in the exudate itself. Although it has since been shown (Billingham & Robinson, 1972) that the substance causing the anti-inflammatory activity in the exudate could be separated from the material responsible for the irritant action, it seemed important to investigate the relationship between these two properties of the plasma fraction used in the present work. The results showed that although the plasma fraction possesses some local irritant activity there was no correlation between this and the anti-inflammatory activity when the two parameters were determined in relation to dose.

A further mechanism by which substances can cause inhibition of local oedemas, such as that produced by carrageenan, may be by inducing a marked, but non-fatal, change in blood pressure. No effects of single or repeated doses of the plasma fraction on the blood pressure of the anaesthetized rat were observed and thus the anti-inflammatory effect was not due either to a hypotensive action or to other changes in the vascular bed.

There have been several reports describing the isolation from inflammatory exudates in man and in experimental animals of substances designated as endogenous anti-inflammatory agents (DiPasquale & Girerd, 1961; Billingham, Robinson & Robson, 1969). These materials are produced in response to an acute inflammatory stimulus and may then inhibit the development of the acute inflammatory reaction both at the original lesion and also at any new sites. The fraction described in this study is present in normal human plasma and may form part of a natural defensive system against inflammation which is always present rather than being produced in response to an inflammatory insult. It is also of interest in connection with the suggestion (McArthur, Dawkins, Smith & Hamilton, 1971) that a natural anti-inflammatory substance exists in human blood and that the clinically useful antirheumatic drugs exert their specific effects in the human rheumatic diseases by interacting with the release of such a substance in the circulation.

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