J. Pharm. Pharmac., 1974, **26**, 878–881 *Received March* 14, 1974

The effects of a human plasma fraction on carrageenan-induced paw oedema in the rat

A. W. FORD-HUTCHINSON, P. N. C. ELLIOTT, J. P. BOLAM AND M. J. H. SMITH

Department of Biochemical Pharmacology, King's College Hospital Medical School, Denmark Hill, London S.E.5., U.K.

A fraction isolated from normal human plasma inhibits the swelling of carrageenan-induced paw oedema in the rat when given as a single intravenous injection at time intervals up to 24 h before and up to 3 h after the administration of the carrageenan. Repeated doses of the fraction cause a delay in the development of the paw oedema.

A fraction showing anti-inflammatory activity in the carrageenan-induced paw oedema reaction in the rat has been isolated from normal human plasma (Ford-Hutchinson, Insley & others, 1973). The method of testing was to give a single dose of the fraction intravenously into a tail vein 30 min before the injection of the irritant into the plantar region of a hind foot. We now describe the effects of administering single and repeated doses of the fraction at various time intervals before and after injection of the carrageenan.

MATERIALS AND METHODS

Plasma fraction. The plasma fraction used was the combined fractions II and IV of Ford-Hutchinson & others (1973), prepared from pooled human plasma (Blood Transfusion Centre, Tooting, London, S.W.17) by ultrafiltration in an Amicon 2L cell under an atmosphere of N_2 using a Diaflo PM 10 membrane followed by elution of the concentrated ultrafiltrate with distilled water after application to a Sephadex G 25 fine column.

Anti-inflammatory testing (Winter, Risley & Nuss, 1962).

Groups of five female albino Wistar rats (Oxfordshire Laboratory Animal Colonies, Southern Ltd.), 150–200 g were used. In the control group each animal received either one or repeated doses of 1 ml of 0·9 g per 100 ml (w/v) NaCl and in the test group each rat received either single or repeated doses of 1 ml of the plasma fraction intravenously into a tail vein. All the injections were filtered through Millipore Millex filter units, type GS 0·22 μm pore size, before being given into a tail vein intravenously at time intervals ranging from 24 h before to 4·5 h after the injection of 0·1 ml of 1·0 g per 100 ml (w/v) carrageenan (Viscarin Marine Colloids) in 0·9 g per 100 ml (w/v) NaCl in the plantar region of the right hind foot. Foot volumes were measured using a mercury plethysmograph (Arnold R. Horwell, Ltd., London) immediately after the injection of the carrageenan (0 h) and at hourly intervals up to 12 h. The results were calculated as percentage increases in the volume of the injected paw compared to the value at 0 h.

Three series of experiments were performed. In the first, a single dose of the plasma fraction was given either 30 min, 3, 6, 9 or 24 h before the carrageenan. In the second, a single dose of the fraction was injected 1, 2 or 3 h after the carrageenan. In the final series, three separate doses of the plasma fraction were given to each rat 30 min before, and 1·5 and 4·5 h after the carrageenan.

RESULTS

Plasma fraction given before carrageenan. Table 1 shows that the injection of the plasma fraction 30 min before the carrageenan caused a significant inhibition of the swelling over 2–6 h. This effect became progressively less pronounced but was still statistically significant during the development of the swelling over 3–6 h when the fraction was given up to 9 h before the irritant. When the fraction was injected 24 h before the carrageenan, a significant inhibition of the paw swelling occurred over 3–4 h only. The results obtained when single injections of 1 ml of saline were given 3, 6, 9 and 24 h before the carrageenan did not differ from those of saline injected 30 min before the irritant.

Table 1. *Anti-inflammatory activity of human plasma fraction when administered at various time intervals before the carrageenan.* Results are given as mean ± standard deviation and expressed as percentage increase in paw volume relative to 0 h. *Significant difference ($P<0.05$) from control. Number of rats tested are in parentheses.

Treatment	Time (h) after carrageenan injection 1	2	3	4	6
Control (10)	26·0±4·3	72·1±12·6	101·6±9·9	105·1±9·9	99·4±10·9
Plasma fraction −30 min (10)	14·6±6·1	25·1±8·6*	29·4±12·7*	52·5±31·2*	67·4±17·3*
Plasma fraction −3h (10)	8·7±4·7	34·7±16·0*	51·0±19·5*	73·2±22·3*	74·6±15·0*
Plasma fraction −6h (10)	12·9±3·0	70·2±22·3	78·2±17·3*	83·4±15·5*	75·7±14·0*
Plasma fraction −9h (10)	13·8±2·8	65·0±12·7	80·7±7·1*	81·0±14·9*	80·5±4·2*
Plasma fraction −24h (10)	12·8±8·7	62·2±18·4	77·9±16·1*	81·4±15·9*	93·2±15·8

Plasma fraction given after carrageenan. Fig. 1 shows that when the plasma fraction was injected either 1, 2 or 3 h after the carrageenan a significant reduction in paw swelling occurred 1 h after the administration of the plasma fraction. The effect became progressively less as the time between the administration of irritant and plasma fraction increased.

Several doses of plasma fraction. The effects of giving a single dose of either saline or plasma fraction and of the injection of three separate doses of either saline or plasma fraction on the development of the carrageenan oedema over a period of 12 h are shown in Fig. 2. Three injections of the saline at −30 min, +1 and +4·5 h did not reduce the development of the paw oedema. A single dose of plasma fraction at −30 min caused a significant inhibition of the swelling over 2–5 h whereas doses at −30 min, + 1 and +4·5 h produced a significantly prolonged reduction in the development of the paw oedema over the period 2–9 h.

DISCUSSION

The results show that the anti-inflammatory activity of the plasma fraction given intravenously against the paw oedema can persist for at least 24 h. This activity

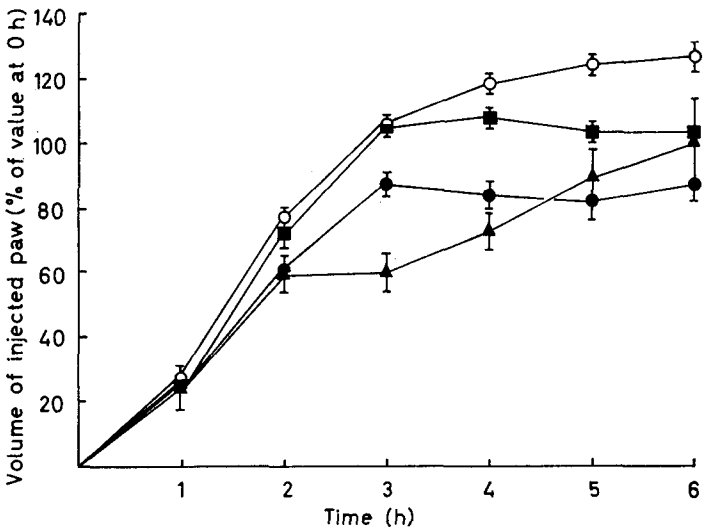

FIG. 1. Anti-inflammatory effects of plasma fraction when administered after the injection of carrageenan. Results calculated as volume of paw as a percentage of the value at 0 h and given as means ±s.e. for the following groups: each of 5 animals; ○——○ saline control, ▲——▲ plasma fraction given 1 h after carrageenan, ●——● plasma fraction given 2 h after carrageenan, ■——■ plasma fraction given 3 h after carrageenan. A statistically significant difference ($P<0.05$) between the saline-treated animals and those given the plasma fraction has been taken to represent a significant anti-inflammatory effect. Such an effect occurred 1 h after the administration of the plasma fraction and persisted for at least 3 h.

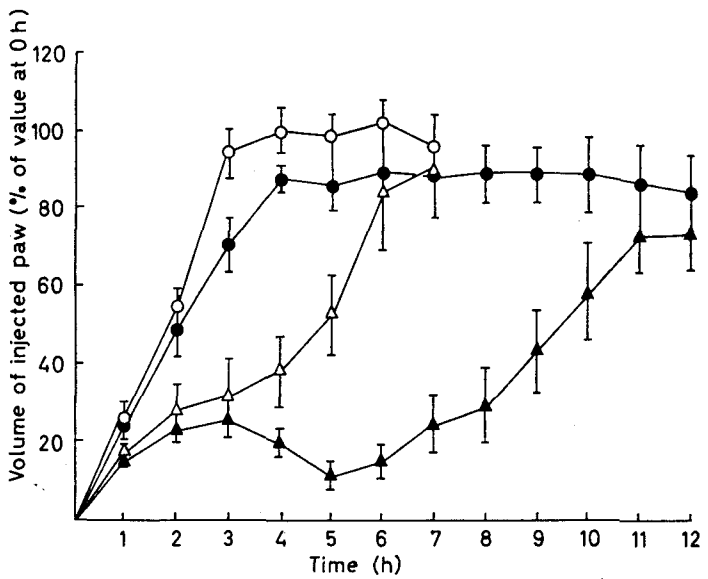

FIG. 2. Anti-inflammatory effects of single and repeated doses of plasma fraction. Results calculated and expressed as in Fig. 1 for the following groups, each of 5 animals; ○——○ single injection of saline at −30 min, △——△ single injection of plasma fraction at −30 min, ●——● three injections of saline at −30 min, + 1·5 h and +4·5 h, ▲——▲ three injections of plasma fraction at −30 min, +1·5 h and +4·5 h. A statistically significant difference ($P<0.01$) was found between the single dose plasma fraction and saline controls at each time interval over the period 2–5 h and between the multiple dose groups at each time interval over the period 2–9 h.

appeared to decline biphasically as the interval between injection of the fraction and the irritant was increased. Thus, if the reduction in paw swelling 3–4 h after the carrageenan is taken as a measure of the relative anti-inflammatory effect of each treatment, then there is a regular decrease in swelling when the fraction was given from −30 min to −6 h before the carrageenan but when given from −6 to −24 h before the irritant the anti-inflammatory activity is essentially the same (Table 1). One possible explanation of these findings is that the active anti-inflammatory substance in the human plasma fraction binds to circulating proteins in the rat but is present in the original 1 ml injection in an amount which exceeds the binding capacity of these proteins. The excess of non-protein bound active substance exerts the most powerful anti-inflammatory effect when given at −30 min, but is largely eliminated by excretory or metabolic processes within a few hours leaving a reservoir of protein-bound active substance to sustain a weaker but more prolonged anti-carrageenan activity in the rat.

The results of the experiments in which the plasma fraction was injected after the carrageenan shows that the development of the paw oedema could be significantly inhibited even when this has been in progress for up to 3 h (Fig. 1). This effect differs from that of conventional anti-inflammatory drugs, such as aspirin, which do not significantly suppress rat pedal oedema if administered after the development of the swelling (Winter & Flataker, 1965; Pircio & Groskinsky, 1966). This finding also suggests that carrageenan-induced paw oedema in the rat is a chronic continuing inflammatory process, at least over several hours, rather than being initiated by a single short-lived event occurring almost immediately after the injection of the irritant (see Di Rosa, 1972).

A single dose of the plasma fraction produces a significant but limited inhibition of the development of the paw swelling over 2·5 h after the administration of the irritant (Fig. 2). This suppression of oedema was both greater and prolonged, i.e. over the period 2–9 h, when repeated injections of the plasma fraction were given at −30 min, +1 and +4·5 h. Thus, if the active substance in the plasma fraction was present before the inflammatory insult and was also given in spaced doses during the period the oedema reached its maximum in the corresponding control animals, then its effects were considerably enhanced. This finding shows that maintaining a sufficient circulating level of the plasma fraction in the rat can produlce a sustained suppression of oedema formation in the carrageenan-induced paw swel ing reaction.

Acknowledgements

We wish to thank Abbott Laboratories Ltd., Beecham Research Laboratories Ltd., the King's College Hospital and Medical School Research Committee, the National Research Development Corporation, the Nuffield Foundation and the Wates Foundation for financial support and the Blood Transfusion Centre, Tooting, London, S.W.17 for the human plasma.

REFERENCES

Di Rosa, M. (1972). *J. Pharm. Pharmac.*, **24**, 89–102.

Ford-Hutchinson, A. W., Insley, M. Y., Elliott, P. N. C., Sturgess, E. A. & Smith, M. J. H. (1973). *Ibid.*, **25**, 881–886.

Pircio, A. W. & Groskinsky, E. J. (1966). *J. Pharmac. exp. Ther.*, **154**, 103–9.

Winter, C. A. & Flataker, L. (1965). *Ibid.*, **148**, 373–379.

Winter, C. A., Risley, E. A. & Nuss, G. W. (1962). *Proc. Soc. exp. Biol. Med.*, **111**, 544–547.