J. Pharm. Pharmac., 1976, **28**, 555–558 *Received December* 9, 1975

Anti-inflammatory actions of dapsone and its related biochemistry

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Dapsone has been examined by two established animal anti-inflammatory models and found to possess anti-inflammatory activity comparable with established non-steroidal anti-inflammatory drugs. Dapsone also possesses some biochemical properties common to other anti-inflammatory drugs.

Dapsone (4,4′-diaminodiphenyl sulphone) is well established clinically as an antileprotic and anti-malarial drug. Recently, McConkey (personal communication) in a clinical trial has reported favourable results when the drug was used in the treatment of rheumatoid arthritis. In addition to his clinical assessment he found that the drug improved the biochemical parameters of the disease such as E.S.R. and Rose-Waaler titres. We have examined the action of dapsone on two animal models used in the study of inflammatory conditions. We have also examined dapsone for biochemical properties which are common to other anti-inflammatory drugs.

METHODS

Animal models used in the study of inflammation

The adjuvant arthritic rat. Arthritis was induced in Wistar strain rats (Newbould, 1963) by the intradermal injection of Freund's adjuvant [5 mg ml^{-1} of finely ground (10 μm) (Best & Christian, 1974) heat killed human strains of tubercle bacillus, kindly supplied by the Ministry of Agriculture Veterinary Laboratories at Weybridge] suspended in liquid paraffin (0·03 ml) into the left hind footpad of each animal. Drugs were administered orally (Newbould, 1963) daily, as a suspension in syrup B.P. The induced arthritis was assessed by measuring the ankle joints of both the injected and non-injected hind feet with a micrometer on the lightly anaesthetized animals every four days.

After the experiment was completed the animals were killed and the major organs examined for abnormalities.

Carrageenan-induced inflammation in the rat

Inflammation was induced by injecting 0·05 ml of carregeenan solution (Winter, Risley & Nuss, 1962) (5% w/v in saline) into the plantar tissue of the left hind paw of the rat. The drugs were administered by intraperitoneal injection 1 h before the carrageenan injection. The induced inflammation was assessed by immersing the foot to the hair line in a mercury bath connected to a pressure transducer linked to a Devices recorder immediately before the injection, and 3 h after.

Biochemical tests on dapsone

Plasma concentrations. These were determined by the colorimetric method of Simpson (1949). Blood was drawn from rats by cardiac puncture and mixed with sodium oxalate and centrifuged in plastic tubes.

Action of dapsone on guinea-pig polymorphonuclear leucocytes (polymorphs). Female guinea-pigs were injected intraperitoneally with 100 ml of saline and the treatment repeated 24 h later, 15 min before the animal's peritoneal cavity was drained by puncture with hypodermic needle. The cells were collected by centrifugation in plastic tubes (600 *g* for 10 min) and washed in saline at 4°. The cells were recovered by centrifuging and finally suspended in 0·34 M sucrose. Portions of the cell suspension were examined and counted by standard techniques employing Leishman's stain and the Neubauer counting chamber. The action of dapsone on the cells was determined by a modification of the method described by Lowe & Turner (1973). Cuvettes filled with 2 ml of the polymorph suspension were placed in the carriage of a spectrophotometer and maintained at 37° by circulating water. Portions (50 μm) of a dapsone solution to give final concentrations over a range 10^{-4}–10^{-6} M in dimethylsulphoxide were added with a microsyringe followed by 10 μl of a 2% v/v solution of Triton X100 in 0·34 M sucrose. The changes in extinction were recorded at 520 nm throughout the experiment. The solutions were gently agitated before each reading. In some experiments dimethylsulphoxide was added in place of the Triton X100 solution.

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Action of dapsone on lysosomes. Liver lysosomes were prepared from freshly excised rat and guinea-pig livers according to (Weissmann, 1965). The action of dapsone (over a final concentration range of 10^{-3}–10^{-7} M) dissolved in dimethylsulphoxide (0·1 ml) on the lysosome suspensions (5 mg protein ml^{-1}) in 0·05 M tris-acetate buffered (0·25 M) sucrose (pH 7·4) were determined by our standard method for measuring the release of acid phosphatase (Lewis, Capstick & Ancill, 1971).

Action of dapsone on human erythrocytes. Dapsone in dimethylsulphoxide (0·1 ml) (to give a final dapsone concentration of 10^{-4}–10^{-6} M) was added to 1 ml of a 1% v/v suspension of erythrocytes in saline. The tubes were incubated for 30 min at 37° and then centrifuged to remove the unbroken cells. The dapsone was omitted from the controls. The supernatants were examined for haemolysis. In other experiments Triton X100 dissolved in saline was added over a wide range of dilutions to the erythrocyte suspension. In these experiments the dapsone (0·1 ml) was added in dimethylsulphoxide solution to 0·5 ml of a 2% v/v suspension of erythrocytes in saline and the Triton X100 solution in saline (0·5 ml) added immediately before the tubes were incubated at 37°, as described above.

Action of dapsone on protein stability. The model employed in previous work was used (Lewis, 1970). Three ml of a 1% w/v bovine albumin solution in saline was incubated for 30 min at 37° after the addition of dapsone (0·1 ml) (to give a final concentration range of 10^{-4}–10^{-7} M) in dimethylsulphoxide solution. The dapsone was omitted from the controls. After incubation the tubes were rapidly cooled to room temperature (60°) and the extinction at 420 nm determined.

RESULTS

Action of dapsone on adjuvant induced arthritis in the rat. The action of dapsone on the development of arthritis in the adjuvant rat is shown in Fig. 1 in which the action of the drug is compared with that of prednisolone. Clearly dapsone has an anti-inflammatory action on adjuvant arthritis at a dose level of 100 and 200 mg kg^{-1} weight. The doses of prednisolone required to produce at least 50% inhibition of the increase in diameter of the injected foot were a tenth of the amount of dapsone required to achieve the same result. A post-mortem examination of the lungs, stomach, spleen and kidneys failed to show any abnormalities induced by dapsone after 21 days treatment.

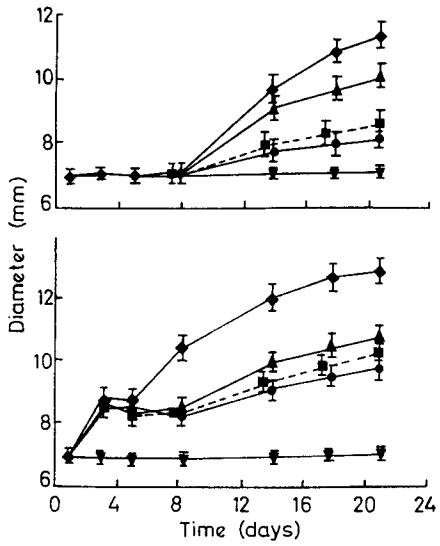

FIG. 1. The effect of orally administered dapsone on adjuvant-induced arthritis in the rat. The upper curve represents the results from the uninjected foot and the lower curve the results from the injected foot. All results are given ± s.e.m. from six animals. ◆ Untreated arthritic rats; ▲ Arthritic rats treated daily with dapsone (100 mg kg^{-1}). ● Arthritic rats treated daily with dapsone (200 mg kg^{-1}). – –■– – Arthritic rats treated daily with prednisolone (200 mg kg^{-1}). ▼ Untreated controls.

Full experiment details are given in the text.

Action of dapsone on carrageenan-induced inflammation in the rat. The action of dapsone on the development of inflammation induced by carrageenan is compared with that of indomethacin in Fig. 2. Both indomethacin and dapsone were

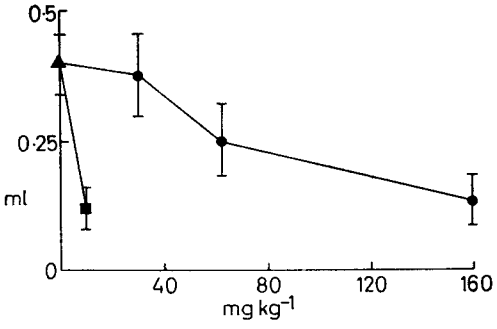

FIG. 2. The effect of dapsone on carrageenan-induced inflammation in the rat. ▲ Control values ± s.e.m. from carrageenan-treated rats with no drug pre-treatment. ● Dapsone treated, ■ indomethacin treated. Full experimental details are given in the text. Ten rats were used in each group. y axis—volume of inflammation (ml). x axis—amount of drug administered (mg kg^{-1}).

effective in inhibiting the development of inflammation but indomethacin was effective at a much lower dose level.

Action of dapsone on erythrocytes. In both sets of experiments employing dapsone alone, and dapsone in the presence of Triton X100, no difference was found in the amount of haemolysis produced in the experimental tubes when compared with the appropriate controls. The effect of dapsone alone was tested over the concentration range 10^{-8}–10^{-2}M, and dapsone at 10^{-5} M had no effect on the cells in the presence of Triton X100.

Action of dapsone on liver lysosomes in vitro. With the rat liver lysosomes the dapsone stabilized the lysosomes over the concentration range of 10^{-4} to 10^{-6} M. Above 10^{-4} M, which is too high to be relevant clinically, the drug labilized the lysosomes (Table 1). A different result was observed with the guinea-pig lysosomes where dapsone stabilized the lysosomes over the entire concentration range examined (10^{-3}–10^{-7} M).

Table 1. *Action of dapsone on liver lysosomes* in vitro. The results represent the % release of acid phosphatase when the control value was adjusted to 100. Values below 100 represent a stabilizing action and values above 100 represent a labilizing action by dapsone. The results are the mean of three determinations.

	% release of acid phosphatase Control value = 100	
Dapsone (M)	Rat liver lysosomes	Guinea pig liver lysosomes
10^{-7}	100	75
10^{-6}	98	42
10^{-5}	15	25
10^{-4}	90	12
10^{-3}	180	4

Action of dapsone on polymorphonuclear leucocytes Dapsone inhibited the lytic action of Triton X100 on these cells and the effect was concentration-dependant (Fig. 3).

Action of dapsone on protein stability. As the concentration of dapsone increased, its stabilizing action against the thermal denaturation of albumin also increased (at concns of M 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4}, the % protein denaturation was respectively 92 ± 0·3, 90 ± 1·0, 88 ± 0·5, 79 ± 2·1; control = 100).

Dapsone concentrations in plasma. The effect of single doses of dapsone and five consecutive daily doses of dapsone are compared in Table 2. Clearly the repeated daily dosing resulted in a build up of its concentration in the blood. Consequently the blood concentrations of the drug in the adjuvant arthritic rat were higher than in the carrageenan experiments since in the former experiment the rats were dosed each day compared with a single dose used in the carrageenan experiment.

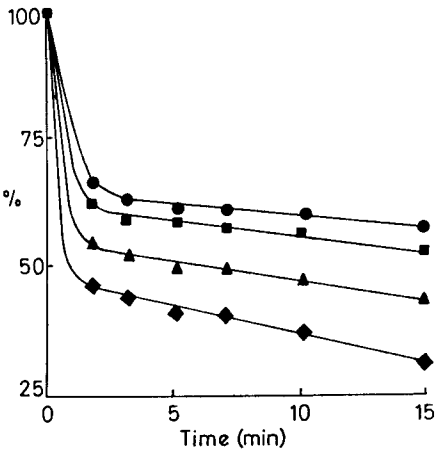

FIG. 3. Action of dapsone on polymorphs. The initial extinction values were adjusted to 100% and the fall in extinction values calculated as percentages of this value. Polymorphs without addition of either Triton X100 or dapsone gave values of 95 ± 2% after 15 min. ● Dapsone 10^{-4} M + Triton X100. ■ Dapsone 10^{-5} M + Triton X100. ▲ Dapsone 10^{-6} M + Triton X100. ◆ Triton X100 alone.

Table 2. *Dapsone concentrations in rat blood.* The results are the mean ± s.e.m. at five determinations.

Amount of dapsone administered at each dose (mg kg^{-1})	No. of doses	Plasma concn of dapsone (μg ml^{-1})
40	One	17 ± 2
100	One	19 ± 3
200	One	21 ± 5
40	Five with 24 h between each	28 ± 8
100	Five with 24 h between each	62 ± 5
200	Five with 24 h between each	92 ± 6

DISCUSSION

Dapsone had an anti-inflammatory effect in both the animal models. Newbould (1963) has reported that a daily oral dose of prednisolone, 20 mg kg^{-1}, is necessary to reduce inflammation by about a half in the adjuvant arthritic rat. Our results are in

agreement with this and we have also shown that an oral daily dose of dapsone 200 mg kg^{-1} is required to produce the same result. The prednisolone and dapsone results are comparable since the human therapeutic dose of prednisolone is about 0·2 mg kg^{-1} and that of dapsone 2 mg kg^{-1}. In contrast the therapeutic concentrations of indomethacin and dapsone are about the same but our results show that indomethacin is about twenty times as effective as dapsone against carrageenan-induced inflammation. This difference may be due to the different blood concentrations between single doses and multiple doses (Table 3), or alternatively the drug may be more effective against the inflammatory mediators producing the arthritis in the adjuvant treated rat than the mediators responsible for the inflammation induced by carrageenan. The biochemical results showed that dapsone shares with many other anti-inflammatory drugs the property of stabilizing lysosomes and polymorphs although the different results obtained for the rat lysosomes when compared to those of the guinea-pig illustrates the caution advocated by Ignarro (1971) in that the properties of a lysosomal preparation depend largely on its source and mode of preparation. The stabilizing action of dapsone on the polymorphonuclear leucocytes may be relevant since these cells accummulate at the site of inflammation (Hersh & Bodey, 1970) and may be responsible for the release of lysosomal enzymes such as proteases (Weissmann, 1969) which have been reported to cause tissue destruction in joint disease (Dingle, 1962). We have also found that dapsone stabilized a protein, which is a property of many other anti-inflammatory drugs (Mizushima, 1964), but whether this property is responsible for the stabilizing action of the drug on membranes it is not yet possible to say. Dapsone at concentrations of physiological interest had no lytic action by itself or in combination with Triton X100 on human erythrocytes. Our results are in agreement with those of Scott & Rasbridge (1973) who suggested that it is a metabolite rather than dapsone itself which has a haemolytic effect on erythrocytes. Under the conditions of our experiments dapsone did not appear to have toxic effects on the rat.

Acknowledgments

The authors thank ICI Ltd and Burroughs Wellcome Ltd for gifts of dapsone and ICI Ltd and the Arthritis and Rheumatism Council for financial support. Miss Anne Powell is thanked for assistance with the biochemical experiments.

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