Br. J. Pharmac. (1974), 52, 609-612

EFFECT OF DRUGS ON RAT PAW OEDEMA INDUCED BY MERCURY

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1 Metallic mercury (0.04 ml) injected into the foot pad of rats induced a consistent inflammatory reaction, which at 4 h showed oedema but no cellular infiltration or vascular changes. The lesions exhibited lymphocytic infiltration, vasodilatation and haemorrhages at 24 and 48 h, and often became cystic after 2-3 weeks, before healing. The oedema volume at 4 h was used to test anti-inflammatory activity of drugs.

2 Cortisone, phenylbutazone, indomethacin, acetylsalicylic acid, flufenamic acid and propranolol exhibited potent, dose-related anti-inflammatory activity. Aminopyrine, chloroquine and chlorpromazine were only moderately effective. Dimercaprol, adrenaline, and to some extent, mepyramine also inhibited mercury-induced oedema.

3 This simple model of acute inflammation may be useful for preliminary tests of anti-inflammatory activity.

Introduction

The pharmacology of mercury compounds has been recently reviewed by Clarkson (1972). However, there is little information available on the fate of metallic mercury deposited in the tissues. We observed that clearance of subcutaneous deposits produced by injections of the metal into the foot pad of the rat, was delayed for days. Initial inflammatory reactions and swelling were inhibited both by dimercaprol and glucocorticoids.

The present work shows that mercury-induced oedema may detect anti-inflammatory activity of drugs given in relatively small doses.

Methods

Male albino rats (165-180 g) of Norwegian strain, maintained on a standard diet and in a room at 25° ± 2°C, were used. They were fasted overnight (water *ad lib.*) before the experiments.

Production of paw oedema by mercury

Mercury (Redistilled, B.P., F.W. Berk & Co. Ltd, London) was shaken 3-4 times with pyrogen-free, sterile distilled water and strained through three layers of sterile, dry gauze. This was repeated until mercury was free of particulate matter and traces of water. The metal was stored in sterile china containers in a cool, dry place.

The hind limb of the rat was thoroughly cleaned. The animal was held with the foot pointing downwards and mercury (0.04 ml) aseptically injected through a No. 23 B.D. needle which was inserted through the skin 1 cm above and then advanced to the middle of the foot pad. The foot volume was measured with a plethysmometer (Singh & Ghosh, 1968), immediately after the injection of mercury and at later intervals. The oedema was assessed by comparing the initial paw volume with subsequent measurements.

Histopathology

Tissue collected by biopsy from the region of mercury deposits was fixed in formolsaline (10%). The sections were stained with haematoxylin and eosin.

Drugs

Cortisone acetate, chloroquine sulphate, chlorpromazine hydrochloride, cyproheptadine hydrochloride, propranolol hydrochloride, lignocaine hydrochloride, mepyramine maleate, phenylbutazone, aminopyrine, paracetamol, 2-bromolysergic acid diethylamide (BOL), (−)-adrenaline, dimercaprol, and sodium salt of penicillin G, were made up fresh in a sterile 0.9% w/v NaCl solution (saline). Solutions (1 mg/ml) of

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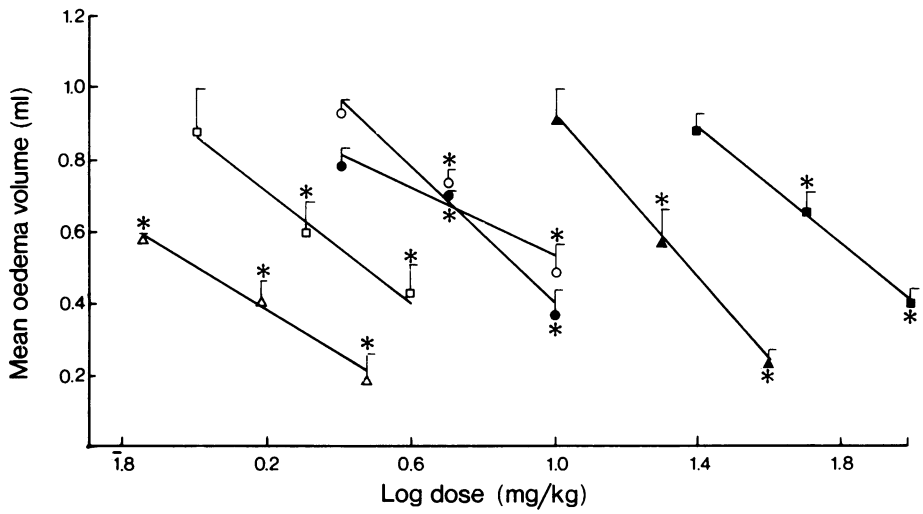

Fig. 1 Oedema induced by mercury in rat paw: dose-effect regression lines of anti-inflammatory activity of cortisone (○) and propranolol (□) given intraperitoneally 30 min before, and of indomethacin (△), flufenamic acid (●), acetylsalicylic acid (■) and phenylbutazone (▲) given orally 60 min before subplantar injections of mercury (0.04 ml/rat paw).

The points are mean oedema values (n = 5) at 4 h, vertical bars represent s.e. mean. In each case, the linearity of regression of effect over the doses was significant ($P < 0.05$); * value significantly different ($P < 0.05$) from the vehicle control (1.172 ± 0.144 ml, see Table 1).

reserpine (Serpasil, Ciba) were prepared immediately before use. Phenoxybenzamine hydrochloride, indomethacin, flufenamic acid, and acetylsalicylic acid were used as suspensions in 2% acacia in water.

Drugs were given orally in 2 ml 60 min before, or intraperitoneally in 1 ml 30 min before mercury was injected into the foot pad. Controls received the corresponding volumes of saline.

Results

Swelling following injection of mercury into the rat foot pad

Swelling appeared soon after the injection of mercury, developed fully in 2-3 h, remained the same for another 2-3 h and then declined to less than half its maximum volume by 24-72 hours. The mean volume of oedema (with s.e. mean) at 4, 24 and 48 h post-injection was 1.134 ± 0.16, 0.74 ± 0.05 and 0.52 ± 0.06 ml, respectively (n = 22). At 4 h, the mean volume of oedema (with s.e. mean) in female rats (0.94 ± 0.06 ml, n = 18) or in rats maintained at 33° ± 1°C (1.02 ± 0.09 ml, n = 8) was not significantly different from the controls. Rats weighing 160 ± 5 g (n = 10) produced a greater degree of oedema (1.3 ± 0.07 ml) than did rats weighing 200 ± 5 g (0.92 ± 0.13 ml, n = 12). In all cases some oedema was still evident after 8 days; at this time the area of the mercury deposit had a pale blue hue, and in 70-80% of rats, cysts containing haemorrhagic fluid were found. The lesions healed 2-3 weeks later, often after the cysts had opened.

During the acute oedema and later, no circulatory occlusion, lymphadenopathy, cellulitis, fever, overt toxicity or death were observed. The rats had little difficulty in using the injected limbs, which showed little tenderness and no rise in temperature.

Histological changes

Four hours after the injection of mercury, the tissue was oedematous; cellular infiltration was minimal or absent and no abnormality was detected in the vascular structure. At 24 h, the tissue showed vasodilatation, few haemorrhages and moderate infiltration of lymphocytes, with a few monocytes and eosinophils. At 48 h, there was definite inflammation, with marked vasodilatation, haemorrhages and an intense lymphocytic infiltration with a few monocytes and eosinophils.

Table 1 Effect of drugs on paw oedema in rats induced by subplantar injection of 0.04 ml of mercury

Drug	*Dose (mg/kg)*	*Route*	n	*Mean oedema volume (ml) ± s.e. mean*	*% inhibition of oedema*
Saline	–	i.p.	22	1.172 ± 0.144	–
(vehicle control)	–	oral	29	1.134 ± 0.122	–
Aminopyrine	40.0	oral	15	0.72 ± 0.16*	36
Chloroquine	10.0	oral	10	0.54 ± 0.026*	52
Chlorpromazine	30.0	i.p.	5	0.56 ± 0.1*	50
Dimercaprol	2.5	i.p.	10	0.306 ± 0.04*	78
Mepyramine	6.0	i.p.	5	0.72 ± 0.036*	37
Adrenaline	0.015	s.c	5	0.62 ± 0.052*†	48
Paracetamol	100.0	oral	5	1.04 ± 0.034	–
Cyproheptadine	30.0	i.p.	5	0.88 ± 0.24	–
BOL	1.0	i.p.	5	0.88 ± 0.07	–
Phenoxybenzamine	10.0	oral	5	0.94 ± 0.026	–

Drugs were given orally 60 min before, or injected (i.p. or s.c.) 30 min before mercury was injected into the foot pad. n = number of rats in the group.
* Differs significantly ($P < 0.05$) from vehicle controls.
† Comparison with i.p. vehicle controls.

Four-hour oedema test; effect of drugs

Findings with five known anti-inflammatory agents suggested the following approximate relative potencies (phenylbutazone = 1): indomethacin 25.4, cortisone 2.7, flufenamic acid 2.4, phenylbutazone 1.0, acetylsalicylic acid 0.3 (Figure 1). It was of interest that propranolol had a potency of 8.6 in this test.

Table 1 shows that aminopyrine, chloroquine and chlorpromazine exhibited anti-oedema activity. Penicillin (10,000 units/kg, i.m., 30 min before, $n = 5$), prior infiltration of the foot pad with 2% lignocaine solution ($n = 5$), or pretreatment with reserpine (3 mg/kg, s.c., on 2 preceding days, $n = 5$) did not affect the mercury-induced oedema.

Discussion

This model of acute inflammation in rats is simple, yet it has several advantages (Weiner & Piliero, 1970). The oedema produced by mercury developed relatively rapidly, and in a single phase, differing from that produced by kaolin (Vinegar, 1968) or carrageenan (Vinegar, Schreiber & Hugo, 1969) which develop in two phases. The single phase is an advantage, because anti-inflammatory drugs may show qualitative and quantitative differences in their actions on the two phases of the oedema. The oedema produced by mercury was reproducible and did not seem to be grossly affected by differences in ambient temperature or sex. The weight of the animals influenced this model less than it did the cotton pellet-granuloma test (Dipasquale & Meli, 1965). Genetic insensitivity, as observed for dextran (Ankier, West, Harris & Luscombe, 1965) was not encountered.

The doses of steroidal and nonsteroidal anti-inflammatory drugs required for inhibition of the oedema were within the limits of tolerance of rats. The model was sensitive enough to detect the activity of indomethacin and chloroquine. The dose-effect relationship for most anti-inflammatory drugs was linear, with slopes at least as good as those obtained by many established screening methods. The relative potencies of anti-inflammatory drugs obtained by the present test agreed with those found by other methods (Winter, Risley & Nuss, 1963; Vinegar, 1968; Sancilio, 1969).

The histological changes induced by mercury at 4 h, resembled those induced by formalin, and differed from carrageenan lesions which show early cellular infiltration. Obviously, the 4 h mercury test is unsuitable for studying leucocyte emigration (DiRosa & Willoughby, 1971). Local tenderness was only mild, which was different from the inflammation produced by formalin, bradykinin or Brewer's yeast. This supports the view that oedema and tenderness may result from different mechanisms (Gilfoil, Klavins & Grumbach, 1963).

The nature of mercury-induced oedema

The volume of mercury used was too small to interfere with drainage of the limb. There were no signs of infection. Experiments with specific

antagonists and reserpine ruled out 5-hydroxytryptamine as a mediator. Histamine may have played a limited role since mepyramine partly inhibited the oedema. The cellular infiltration at 4 h was only slight which suggested that prostaglandins were of little importance (DiRosa & Willoughby, 1971). It was interesting that the oedema was suppressed by adrenaline and propranolol, but not by phenoxybenzamine or by pre-treatment with reserpine. Whilst anti-oedema activity of adrenaline has been reported in various tests (Brown & West, 1965; Vinegar, 1968), the effect of propranolol was unexpected; indeed, this drug inhibits anti-inflammatory actions of other agents (Riesterer & Jaques, 1968). The anti-inflammatory effect of propranolol could not be attributed to its local anaesthetic action, because lignocaine did not suppress the oedema.

Since dimercaprol antagonizes the action of a metal only if a mercaptide is formed with essential cellular sulphydryl groups (Levine, 1965), mercury appears to produce oedema by inhibiting sulphydryl groups, probably after it has been locally oxidized to a mercuric rather than a mercurous ion (Clarkson, 1972).

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(Revised January 28, 1974)