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THE EFFECTS OF METIAMIDE ON CELL-MEDIATED IMMUNE REACTIONS IN THE GUINEA-PIG

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1 The effects of the H_2-receptor antagonist, metiamide, on two types of cell-mediated immune response *in vivo* was investigated in the guinea-pig, in a test of the hypothesis that H_2-receptor antagonists would result in 'runaway' unregulated hypersensitivity reactions.

2 Metiamide in doses of 5 mg–125 mg/kg given by 6-hourly injection during sensitization and challenge did not modify the delayed hypersensitivity reaction to tuberculin.

3 Metiamide in doses of 25–125 mg/kg given by injection, 6-hourly throughout sensitization to dinitrofluorobenzene (DNFB) and subsequent challenge six days later, did not modify the contact sensitivity reaction to DNFB.

4 It is concluded that metiamide, given during these cell-mediated immune reactions in the guinea-pig, does not enhance cell-mediated hypersensitivity responses.

Introduction

It has recently been suggested that histamine, along with other endogenous pharmacologically active agents, may act *in vivo* to regulate the character and intensity of immunological and inflammatory responses (Bourne, Lichtenstein, Melmon, Henney, Weinstein & Shearer, 1974). Evidence has been put forward that, *in vitro*, histamine inhibits several processes involved in these responses such as direct lymphocyte-mediated cytotoxicity (Henney, Bourne & Lichtenstein, 1972), production of macrophage inhibitory factor (MIF) (Rocklin, 1976), lymphocyte activation (Rocklin, 1976; Beets & Dale, unpublished results), and histamine release from basophils (Bourne, Melmon & Lichtenstein, 1971; Lichtenstein & Gillespie, 1973). Rocklin (1976) has also claimed that histamine reduces delayed hypersensitivity reactions *in vivo*. The proposition, in general, is that histamine regulates immunological and inflammatory reactions, by acting on H_2-receptors on the cells concerned, resulting in an accumulation of cyclic adenosine 3,'5'-monophosphate (cyclic AMP), which inhibits or 'turns off' several cell functions. It is suggested that in doing so it serves 'to protect the host from the dangerous consequences of an unregulated immune response' (Bourne *et al.*, 1974).

If this is indeed so, one possible corollary could be that H_2-receptor antagonist drugs such as metiamide and cimetidine would exaggerate immunological and inflammatory reactions by preventing the suggested modulatory action of histamine. H_2-receptor antagonists are proving to be valuable in the treatment of peptic ulcer, Zollinger-Ellison syndrome, and other gastro-intestinal conditions (Thjodleifsson & Wormsley, 1975; Multicentre trial, 1975; Richardson & Walsh, 1976; MacDonald, Steele & Bottomley, 1976; Black, 1976). One of them, cimetidine, has recently been released for clinical use in this country and is likely to be widely prescribed. It is of considerable importance to know whether this group of drugs has a hidden potential for exacerbating any concurrent or intercurrent conditions with an inflammatory and/or anti-allergic component.

The present study attempts to determine whether such a hidden potential exists by examining the effect of a range of doses of metiamide on two animal models of cell-mediated immune reactions: the tuberculin reaction in the guinea-pig (the prototype of delayed hypersensitivity reactions) and contact sensitivity.

Methods

Delayed hypersensitivity tests

Eighteen female Hartley guinea-pigs were sensitized with complete Freund's adjuvant (CFA) and subsequently challenged with tuberculin (PPD).

Sensitization. Dried killed tubercle bacilli (10 mg) was mixed into 10 ml of complete Freund's adjuvant on a Whirlimixer. Each guinea-pig received 0.4 ml of this preparation injected into 3 sites: 0.2 ml intramuscularly into the back of the neck, 0.1 ml in-

tradermally into the skin of the right ear and 0.1 ml intradermally into the skin of one hind foot.

Challenge. The skin was shaved on both flanks and 0.1 ml of tuberculin was injected intradermally, 5 μg on the left side and 50 μg on the right side.

Drug treatment. The 18 animals received the following subcutaneous injections in 1 ml/kg: Group I (5 animals) 0.9% w/v NaCl solution (saline); Group II (4 animals) metiamide 5 mg/kg; Group III (4 animals) metiamide 25 mg/kg; Group IV (5 animals) metiamide 125 mg/kg. The schedule of drug treatment was as follows: injections were started 24 h before sensitization and continued at 6-hourly intervals for 4 days subsequently. Two days before challenge the 6-hourly injections were started again and continued until the skin reactions had been read.

A nineteenth animal served as a 'blank' control, i.e. it was not injected with saline or metiamide and it was not sensitized with CFA but it was challenged with tuberculin at the same time as the others.

Assessment of skin reactions. At 24 h, erythema and skin thickness were measured in 'blind' conditions so as to avoid subjective biasing of the results. Two measurements of the diameter of the erythema were taken at right angles to each other with calipers, and the mean diameter calculated. Skin thickness was measured with Snelltaster skin calipers. Two measurements of normal skin thickness were taken on either side of the delayed hypersensitivity reaction, and the skin thickness measured at the actual site of the reaction was expressed as the % increase above the mean thickness of these two measurements.

Contact sensitivity tests

Seventeen Hartley guinea-pigs were sensitized with dinitrofluorobenzene (DNFB) and subsequently challenged with DNFB 7 days later.

Sensitization. Fifty μl of 10% DNFB in a mixture consisting of equal parts of acetone and olive oil was applied to the right ear of each guinea-pig.

Challenge. The animals were shaved on both flanks and 4 areas marked out for treatment. Four different concentrations of DNFB were used: 0.05%, 0.1%, 0.25% and 0.5%, made up in a mixture of four parts of acetone to one part of olive oil. At each site 20 μl of the requisite solution was applied with an Eppendorf micropipette.

Drug treatment. The animals received the following injections: Group I (5 animals) saline; Group II (5 animals) metiamide 25 mg/kg; Group III (5 animals) metiamide 125 mg/kg. Injections were started 24 h before sensitization and continued at 6-hourly intervals for 5 days. On the fifth and sixth day they were given at 12-hourly intervals, then 6-hourly injections were recommenced and continued for a further 3 days.

Group IV (2 animals) received one injection of cyclophosphamide, 300 mg/kg intraperitoneally, three days before sensitization.

Assessment of skin reactions. The reactions to locally applied DNFB were rated on a 4-point scale (Turk, Parker & Poulter, 1972). All ratings were done 'blind'.

Drugs

The following drugs were used: 1-fluoro-2,4-dinitrobenzene (Hopkin & Williams); cyclophosphamide (Koch-Light Laboratories); carboxymethyl-cellulose (sodium salt) (Sigma Chemical Co.); Freund's Complete Adjuvant (Difco Laboratories); metiamide (S.K. & F.); *M. tuberculosis,* heat killed strains C, DT and PN (Ministry of Agriculture, Fisheries and Food Central Veterinary Lab., Weybridge, Surrey).

Results

Delayed hypersensitivity reactions to tuberculin

The intensity of the delayed hypersensitivity reactions was assessed 24 h after challenge by measuring both the percentage increase in skin thickness and the diameter of the erythematous lesion at the site of the tuberculin injection. The results are given in Figure 1a (i) and (ii). The control guinea-pig showed no reaction at all at the injection site. All other animals showed a dose-related reaction to the injections of tuberculin, but there was no difference between the control group and the three groups treated with different doses of metiamide.

The dosage schedule chosen was based on preliminary experiments carried out by Caldwell (unpublished results). These showed that metiamide made up as a 10% solution in 0.5% carboxymethyl-cellulose and injected subcutaneously into the flank, gave fairly high blood levels for at least 6 h; for example, a dose of 250 mg/kg, produced the following mean blood levels (μM, with s.e. mean): at 2 h 224 ± 36; at 4 h 151 ± 17; at 6 h 69 ± 5; at 8 h 16 ± 6. Similar results were obtained when injections were made into the scruff of the neck.

In the tuberculin experiments of the present study, injections of metiamide or saline were started 24 h before sensitization and were carried on for 4 days i.e. during the initial critical phase of the immunological response to antigen. Injections were also given 6-hourly for 24 h before challenge and during the 24 h after challenge, up to the time when skin reactions were measured.

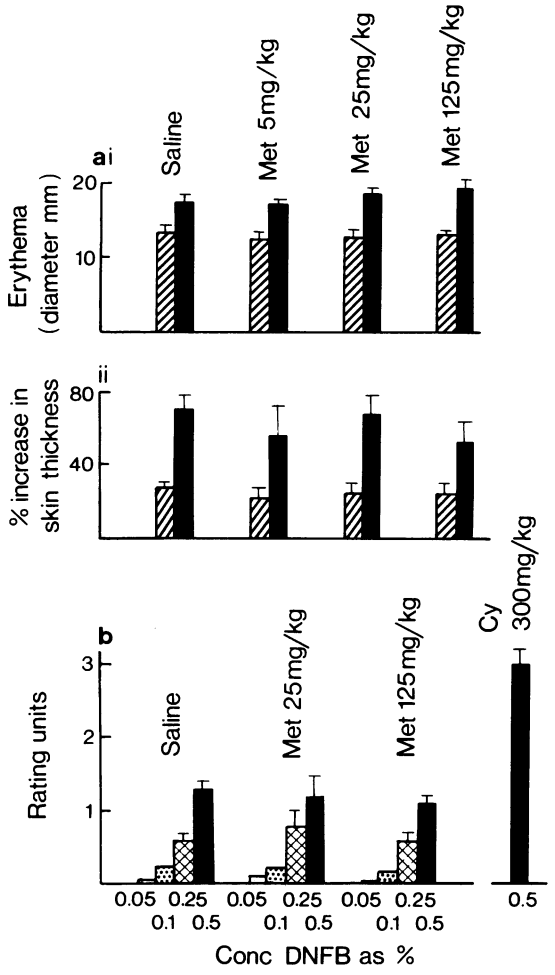

Figure 1 Cell-mediated skin reactions in the guinea-pig. (a) Skin reactions to two doses of tuberculin in 4 groups of guinea-pigs injected with saline or the indicated doses of metiamide (Met) as described in the methods section. Means are given; vertical lines show s.e. mean. All 4 groups had been sensitized to tubercle bacilli fourteen days before challenge with tuberculin. Measurements were made 24 h after challenge. Hatched columns, 5 μg tuberculin; solid columns, 50 μg tuberculin. (i) Diameter of erythema (mm). (ii) Induration of lesions given as % increase in skin thickness as compared with normal skin. (b) Contact sensitivity reactions to 4 doses of dinitrofluorobenzene (DNFB) in 4 groups of guinea-pigs injected with saline or metiamide (Met), or with one dose of cyclophosphamide (Cy) (see methods section). Means are given; vertical lines show s.e. mean. All groups had been sensitized with DNFB 7 days before challenge. The degree of reaction was rated on a 4-point scale 24 h after challenge.

Similar results were obtained when metiamide, 5 mg to 125 mg/kg was given at 6-hourly intervals during sensitization but not during challenge, or during challenge but not during sensitization.

Contact hypersensitivity reactions

The skin reactions to DNFB were rated on a 4-point scale (Figure 1b). All animals showed a dose-related response to the challenging doses of DNFB. When the results were analysed by t tests, there was no significant difference ($P > 0.05$) between the reactions of the control group and those of the groups receiving metiamide. Metiamide had not therefore produced an increase in DNFB-induced contact hypersensitivity.

The lesions produced by challenge with a given concentration of DNFB were not the maximum possible reactions, i.e. the dose-response curve could be shifted to the left. This was demonstrated by the marked increase in the local reaction produced by pretreatment of the animals with cyclophosphamide (Turk *et al.*, 1972).

Discussion

The tuberculin reaction is the prototype of cell-mediated delayed hypersensitivity reactions (Dvorak, 1974; Turk, 1967). The key cells in these reactions are considered to be the T-lymphocytes which in the presence of antigen transform and secrete a variety of mediators or lymphokines (such as MIF) which influence the activities of other cells including B-lymphocytes, macrophages, etc. (see review by Dvorak, 1974). The activated lymphocytes may also have direct cytotoxic effects. Both T and B-lymphocytes are purported to have or to develop on exposure to antigen, H_2-receptors (Henney *et al.*, 1972; Melmon, Bourne, Weinstein, Shearer, Bauminger & Kram, 1974), and thus to become susceptible to histamine which, produced locally during the reaction, is thought to modulate the response by a type of negative feedback (Bourne *et al.*, 1974). An H_2-receptor antagonist could thus be expected to prevent this negative feedback and lead to a runaway unregulated response. The presence of metiamide in the plasma during the critical phase of sensitization and challenge should therefore increase the tuberculin response in a dose-related fashion. In the present study there was no difference between the control animals and the metiamide-treated animals.

Contact sensitivity is another cell-mediated delayed hypersensitivity reaction, and the key cell is also believed to be a T-lymphocyte. Originally characterized as being cell-mediated by Landsteiner & Chase (1942), contact sensitivity is now included by some workers in the category of cutaneous basophil hypersensitivities (Dvorak, Simpson, Bast & Leskowitz, 1971; Dvorak & Dvorak, 1975), because one of the

main phenomena is a local accumulation of basophils. In the guinea-pig, basophils may constitute 15 to 34% of the total infiltrating cells, which is all the more remarkable when it is considered that they are otherwise a relatively rare cell type, forming only 0.5% of circulating leucocytes. Basophils have a high content of histamine, which is released during immediate hypersensitivity reactions. Furthermore, basophils have been reported to have H_2-receptors which on stimulation modify histamine release (Lichtenstein & Gillespie, 1973). If this is the case, the effect would be altered by H_2-receptor antagonists. Metiamide should, in dose-related fashion, increase the intensity of the skin reactions. However, this was not found in the present study.

In the contact sensitivity experiments, a 'positive' control group was included, viz. guinea-pigs pretreated with cyclophosphamide, which is known to produce increased skin reactions. It has been suggested by Turk *et al.* (1972) that the cyclophosphamide depletes B-lymphocytes which would normally have a suppressive effect on the reaction. It is of interest that in animals pre-treated with cyclophosphamide no basophil infiltration in the contact sensitivity lesions was found (Katz, Heather, Parker & Turk, 1974).

It thus appears that metiamide does not exacerbate either tuberculin hypersensitivity or contact sensitivity in the guinea-pig even when it is continuously present at high concentration in the plasma. This does not necessarily mean that histamine is not an important regulator of these reactions, but its action probably involves factors other than negative feedback by stimulation of H_2-receptors.

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