

The Effect of Cyclophosphamide on the Toxic Contact Reaction to Croton Oil in Guinea Pig

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Cyclophosphamide is a cytostatic agent used clinically and in experimental studies for its immunosuppressant effect. Effects on the afferent and efferent limb of the contact allergic reaction have been shown. The substance has also non-specific, anti-inflammatory effects and these were investigated in this paper by studying the effects of single doses of cyclophosphamide on the toxic contact reaction to croton oil (a non-specific inflammatory reaction) in guinea pig. Counting of the dermal cellular infiltrate showed that mononuclear and granulocyte counts decreased in a dose-dependant fashion after cyclophosphamide administration. Erythema and oedema in the test reactions decreased generally, although at the highest dose a paradoxical increase was seen. *Key words: Immunosuppressant; Cytostatic; Cellular infiltrate.* (Received December 14, 1984.)

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Some cytostatic agents, notably cyclophosphamide, methotrexate and azothioprine have effects on immunologic reactions (1, 2, 3, 4). They are then often referred to as immunosuppressant agents. Since however these agents often also have non-specific anti-inflammatory effects it can be difficult to be certain that an effect observed represents true immunosuppression (5). Using a method for quantification and differentiation of the dermal inflammatory infiltrate in the contact allergic reaction to oxazolone (6), we have shown that cyclophosphamide has marked effects on both cell infiltrates and the macroscopic appearance of tests when administered in a single relatively high dose (300 mg/kg) intraperitoneally at different times prior to test (7). Given three days prior to first test day, erythema and oedema of the reaction diminished as did the mononuclear, basophil and eosinophil dermal cell infiltrates. The aim of this study was to assess the effect of cyclophosphamide in the same dose administered at the same time prior to test on a non-specific contact reaction, the toxic contact reaction to croton. This reaction gives a relatively uniform degree of erythema and oedema macroscopically and a predominantly mononuclear infiltrate which, like the macroscopic score, is maximal at 24 to 48 hours. To establish dose effects, smaller doses of cyclophosphamide would also be given.

MATERIALS AND METHODS

Animals. Female albino Dunkin Hartley guinea pigs weighing about 300 g at the time of sensitization were kept under standardized conditions. The flanks are shaved with an electric razor prior to testing.

Testing. 10 μ l of a 0.1% croton in oil preparation is applied to 1 cm² areas on the flanks. Macroscopic assessment was performed prior to sacrifice (0 = no change or uncertain reaction, + = redness, ++ = redness with slight palpable induration and +++ = redness and obvious swelling). A "macroscopic score" is obtained by dividing the total number of "+" for the group by the number of animals in the group. Although strict statistical analysis of changes in the macroscopic score is not possible, we designate changes in the mean score as "slight" (change by 0.3-0.4), "clear" (change by 0.5-0.9) or "marked" (change by >1.0).

Histology. After sacrifice, punch biopsies of the test site and normal skin are taken, fixed in 10% neutral, phosphate buffered formalin, embedded in glycol methacrylate and polyethylene glycol

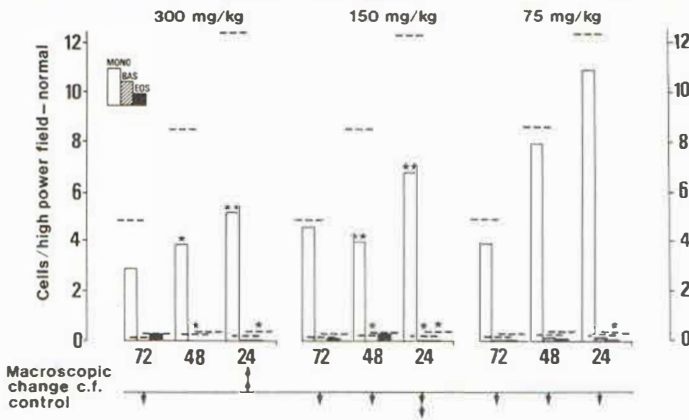


Fig. 1. The effect of cyclophosphamide on the toxic contact reaction to croton oil. Cyclophosphamide was administered in a single dose (300, 150 or 75 mg/kg) three days prior to first test day. Columns show the cell responses (test count minus normal) for mononuclear, basophil and eosinophil cells at 72, 48 and 24 hours (the order in which the tests were applied after cyclophosphamide administration). Control values are shown by---. Statistical significance of changes is shown (* $p < 0.05$, ** $p < 0.01$). The size and direction of changes in the macroscopic score compared to the control group are shown schematically by arrows (\uparrow = slight, [\uparrow] = clear, for details see Methods).

(Sorvall Embedding Medium, Du Pont USA), cut in 3 μ m sections and stained with May-Grünwald-Giemsa. Microscopic assessment is performed with a 1000 \times oil immersion lens.

Counting. 20 fields just below the dermo-epidermal junction are counted and results presented as the average number of cells per high power field. Granulocytes are differentiated on morphological grounds into basophils, eosinophils and neutrophils. Mononuclear cells (small and medium-sized lymphocytes, monocytes) are counted as a single group. Mast cells are also counted. All cell types are included in a "total" cell count. In every animal the dermal cellular infiltrate in normal skin is counted. This figure is subsequently subtracted from the test area counts, giving a "test minus normal count" for every animal and cell type. Mean results for the group are then calculated and values are statistically analysed by paired *T* test against an internal control group and a larger pooled control group.

Experimental design. A single intraperitoneal dose of cyclophosphamide (300, 150 or 75 mg/kg) was administered to three groups of animals three days prior to first test day. Tests were applied 3, 2 and 1 days prior to sacrifice to give a 24, 48 and 72 hour reaction. 7 to 10 animals were in each dosage group with 1 or 2 control animals in each group.

Table I. The effect of cyclophosphamide on the toxic contact reaction to croton oil

Macroscopic scores and the dermal inflammatory cell response (test count minus normal) for total (TOT), mononuclear (MONO), basophil (BAS), eosinophil (EOS) and neutrophils (NEUT) in cells per high power field (average of 20 fields) are shown for the control reaction and groups given cyclophosphamide in a single dose (300, 150 or 75 mg/kg) three days prior to first test day

	24 hours					
	Macroscopic score	Tot	Mono	Bas	Eos	Neut
Control group (n=6)	2.0	13.8	12.4	0.2	0.35	0.75
Cyclophosphamide, 300 mg/kg (n=5)	2.7	5.4	5.2	0.02	-0.02	0.15
Cyclophosphamide, 150 mg/kg (n=7)	1.1	7.4	6.8	0.03	-0.02	0.12
Cyclophosphamide, 75 mg/kg (n=8)	1.5	11.5	10.9	0.16	-0.03	0.3

RESULTS

The inflammatory infiltrate in normal untested skin did not differ from our control material. Cell infiltrates in the untreated sensitized control animals ($n=6$) had slightly fewer basophils but otherwise did not differ from the larger pooled control group ($n=53$). The macroscopic score responded in a biphasic, dose-related fashion. The 300 mg/kg dose worsened the macroscopic appearance of the 24 hour reaction. At 48 and 72 hours for the 300 mg/kg dose and for all points in the 150 mg/kg and 75 mg/kg group the macroscopic score diminished. The degree of change is indicated in Fig. 1.

Table I shows the cellular response (test count minus normal) and the mean macroscopic scores for the control reactions and the three doses of cyclophosphamide used. Total cell counts fell for all but the lowest (75 mg/kg) dose of cyclophosphamide. The infiltrate in this model is predominantly mononuclear and this count fell at all points. Changes were statistically significant for the 24 hour and 48 hour reactions at the higher doses (Fig. 1). Counts for basophils, eosinophils and neutrophils also fell (Table I, Fig. 1). The basophil count fell significantly ($p<0.05$) in the 48 hour reaction for the 300 and 150 mg/kg doses of cyclophosphamide. Eosinophil counts were significantly reduced ($p<0.05$) in the 24 hour reaction at all cyclophosphamide doses.

DISCUSSION

Cyclophosphamide had clear effects on the dermal cellular infiltrate of the toxic contact reaction to croton oil. The total and mononuclear cell infiltrate counts were significantly diminished for the 300 mg/kg and 150 mg/kg doses. The lowest dose (75 mg/kg) did reduce total and mononuclear cell counts but changes were not statistically significant.

Clear effects were also exerted on granulocytes, with reduction in counts at most points, although statistical significance was not reached at all points due to the small number of cells involved and the relatively small sample sizes. The tendencies are however clear and the lowest dose of cyclophosphamide (75 mg/kg) still had effects on granulocytes. Whilst all significant changes in cell counts were reductions, the macroscopic score showed a biphasic response. Although erythema and oedema in general decreased, the 24 hour reaction at the top cyclophosphamide dose showed a clear increase in macroscopic score, despite the fact that the cell counts had decreased.

Thus, cyclophosphamide administered in a dose of 300 mg/kg three days prior to first test day showed effects on the toxic contact reaction to croton oil (decreased oedema and

48 hours						72 hours					
Macroscopic score	Tot	Mono	Bas	Eos	Neut	Macroscopic score	Tot	Mono	Bas	Eos	Neut
1.2	9.0	8.5	0.25	0.35	0.07	0.8	5.5	4.8	0.19	0.24	0.17
1.4	4.2	3.9	0.01	0.01	0.04	0.4	3.6	2.8	0	0.27	0.05
0.8	4.9	3.9	0.04	0.32	0.37	0.4	4.9	4.5	0	0.1	0.05
0.9	8.1	7.9	0.13	0.09	0.06	0.5	4.3	3.9	0.05	0.1	0

erythema and falls in the mononuclear, basophil and eosinophil dermal cell infiltrates) which were similar to those seen in our previous study of the effects of cyclophosphamide on the allergic contact reaction to oxazolone (7). In the present study, smaller doses were also given and this showed that the cellular effects are dose dependent and that granulocytes are more sensitive than mononuclear cells. The paradoxical increase in erythema and oedema seen in the 24 hour reaction in the present experiment is similar to an effect seen at some points in our allergic contact reaction study. It has been suggested that such increases in erythema and oedema may be due to shifts toward more immediate types of allergic reactions, possibly basophil mediated ones. In the toxic contact reaction since no sensitization is involved, the mechanism must lie at a level involving a much more direct effect on inflammation perhaps via release of mediators.

Tests were performed 3 to 6 days after the administration of cyclophosphamide. During this period, the peripheral blood is leukopenic (7). Whilst this may explain the poor recruitment of cells to the dermal reaction, it is not necessarily the only explanation since in our work on the effect of cyclophosphamide on the efferent limb of the contact allergic reaction, we found at some points pronounced cell infiltrates despite peripheral leukopenia (7). Cyclophosphamide may well have direct effects on mediators of inflammation and on the mechanism of recruitment of cells to a cutaneous inflammatory reaction.

Parker & Turk (9) found that cyclophosphamide had no effect on the reaction to intradermal injection of a 1:10 dilution of turpentine (used to induce a non-specific inflammatory reaction). Our studies on the toxic contact reaction show that cyclophosphamide exerts effects on both the macroscopic appearance and the dermal inflammatory infiltrate of the reactions. These effects are similar in nature to those seen in previous studies of the allergic contact reaction in the same dose, timing and experimental model and are dose dependant. Consequently when cyclophosphamide is used as a tool in the investigation of pathogenetic mechanisms in contact allergy and delayed hypersensitivity in general, due consideration must be taken to this substance' clear non-specific anti-inflammatory effects.

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