Acta Dermatovener (Stockholm) 61: 307–311, 1981

SUPPRESSION OF DNCB-INDUCED IRRITANT DERMATITIS BY COLCHICINE

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Abstract. In vivo effects of colchicine on DNCB-induced primary irritant dermatitis in guinea-pigs were investigated with simultaneous evaluation of *in vitro* chemotactic activity of polymorphonuclear leukocytes (PMNs). Colchicine, 125, 250 and 500 μg/kg, was injected intraperitoneally 1 hour prior to the painting of 20 μl of 10% DNCB in acetone on shaved dorsum of the animals. In the control animals, erythema and induration were maximum and dermal PMN infiltration was most intense at 24 hours. In the colchicine-treated animals, induration—but not erythema—was reduced, with sparse dermal PMN infiltration. *In vitro* chemotaxis of PMNs from cardiac blood to LPS-treated serum using Boyden chamber techniques revealed maximal suppression of the activity in the colchicine injected animals at 12 hours. It is suggested that the reduced induration and decreased infiltration of PMNs in the inflammatory tissue in the colchicine-injected animals are caused by prior suppression of locomotion of PMNs in the peripheral blood.

Key words: Colchicine; DNCB dermatitis; Polymorphonuclear leukocytes; Chemotaxis

Colchicine, a unique anti-inflammatory agent, has long been used in gouty arthritis, providing a dramatic relief of acute attacks. The drug also induces clinical improvement in necrotizing vasculitis (5), and of skin manifestations of Behcet's disease (9). It has been shown that PMNs play an important role in the pathogenesis of these three diseases. Suppression of inflammatory responses by colchicine has also been shown in animal experiments. A typical example of such suppression is observed in the reversed passive Arthus reaction (2). Furthermore, a single injection of colchicine markedly inhibits the ability of rats to mobilize PMNs into the peritoneal cavity stimulated by bacterial endotoxin (4). In guinea pigs, intraperitoneal colchicine reduces the PMN response in the lesions induced by intradermal inoculations of staphylococci. The larger lesions follow in such animals compared with those in untreated animals, indicating that this is due to an initial delay in the delivery of phagocytes necessary for normal host defence (7).

Among a variety of impairments of PMN functions exerted by colchicine, Wallace, Omokoku & Ertel (11) have suggested that interference with chemotaxis is indeed the way whereby colchicine in therapeutic doses influences the PMNs.

In vitro studies on the effects of colchicine on cell locomotion indicate that dysfunction of microtubules accounts in part for colchicine-induced inhibition of *in vitro* chemotaxis of PMNs (8).

We investigated the *in vivo* effects of colchicine on 1 chrolo 2:4 dinitrobenzene (DNCB)-induced primary irritant dermatitis which is characterized by massive dermal infiltration of PMNs. This predominant PMN infiltration is explained as being due to the production of a chemotactic factor for PMNs in the lesion following the application of DNCB (1). Simultaneous evaluation of the *in vitro* chemotactic activity of PMNs suggested that a decrease in infiltration of PMNs into the lesions of colchicine-treated animals resulted from inhibition of directional chemotaxis.

MATERIALS AND METHODS

Animal

Albino guinea pigs (outbred Hartley strain), weighing 450–500 g, were used. Three animals in histological examinations and 6 animals in chemotactic assay were included in each experimental group.

Colchicine treatment

Colchicine (Sigma Chemical Co., St. Louis, Mo) was freshly diluted with physiological saline at a concentration of 250 μg/ml. Animals were administered a single intraperitoneal (i.p.) injection of this solution to give a final doses of 500, 250 or 125 μg/kg of colchicine 1 hour before the application of DNCB. Control animals were given saline i.p.

DNCB-primary irritant dermatitis

20 μl of a 10% acetone solution of DNCB was applied to an area (about 4 cm^2) of the clipped dorsum of the trunk. The intensity of dermatitis was scored macroscopically as given in Table 1.

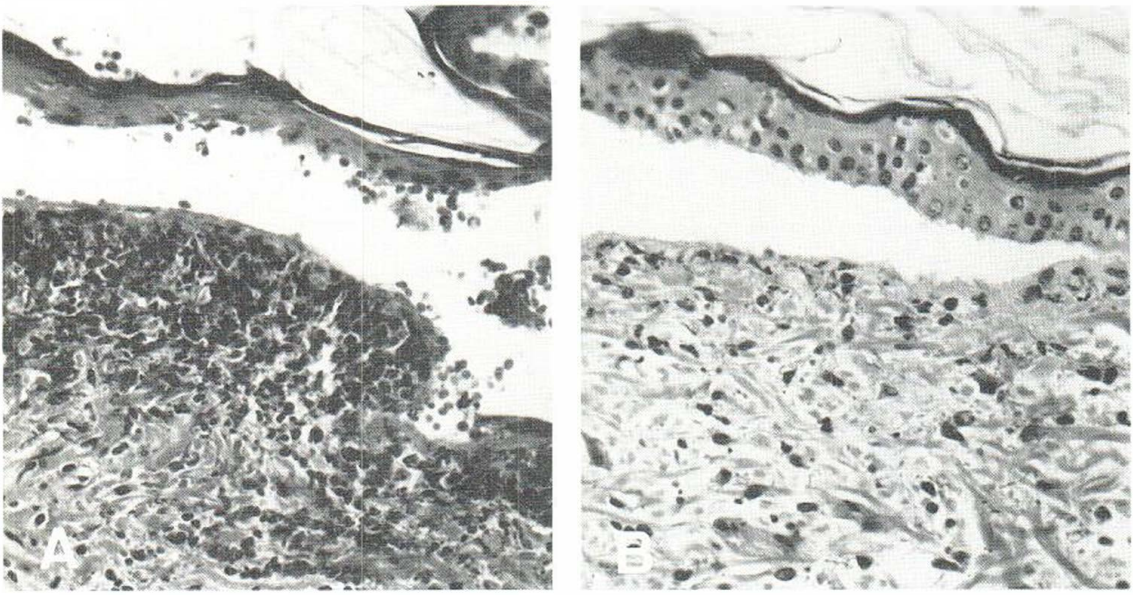

Fig. 1. Dermal PMN infiltration and epidermo-dermal separation 24 hours after the application of 10% DNCB solution (H & E ×180). (A) Control; (B) colchicine-treated animal (250 μg/kg).

Histology

Skin biopsies from DNCB-treated sites were obtained at 2, 12 and 24 hours with a 4 mm punch. The specimens were fixed in formalin and stained with H & E. The number of PMNs and mononuclear leukocytes (MNLs) in dermal infiltration was counted in an area of 0.04 mm^2 in each of 10 sections per one specimen.

In vitro chemotactic assay

PMN chemotactic activity was determined by using modified Boyden chambers as previously described by Tagami & Ofuji (10). Serum activated by lipopolysaccharide (LS) (*E. coli*; 026: B6, Difco Lab., Detroit, Michigan) was used as a chemotactic factor. Blood PMNs from the animals at 2, 12 or 24 hours were suspended in Hanks' balanced salt solution at a concentration of 2×10^6/ml. The cell suspensions were placed in the upper compartment of the chambers and were incubated in a humidified atmosphere with 5% CO_2 at 37°C for 3 hours. Millipore filters were fixed in methanol, stained with hematoxylin and mounted on a glass slide with the test chamber side uppermost. For each filter, 10 random fields were counted at a magnification of ×400 and the number of PMNs migrating completely through to the bottom surface of the filter was recorded. Chemotactic activity was expressed as the average number of cells per high-power field.

WBC count

The effect of colchicine on total counts and per cent of PMNs of peripheral leukocytes was examined before and 24 hours after colchicine injection.

Table 1. *Scoring of degrees of erythema and induration*

	Number of animals	2 hours		12 hours		24 hours	
		Erythema[a]	Induration[b]	Erythema[a]	Induration[b]	Erythema[a]	Induration[b]
Control	1	–	–	+–	++	+–	+–+
	2	–	–	++	++	++	+–+
	3	–	–	+–	–+	+–	+–+
Colchicine treated (250 μg/kg)	1	–	–	+–	+	+	+
	2	–	–	++	–	+	+
	3	–	–	++	+	++	++

[a] Scored into 4 degrees: (–); none, (+); slight, (++); moderate, (+++); pronounced.
[b] Scored as follows: (–) not elevated; (+) raised less than 1 mm above the adjacent surface of the skin; (++) raised less than 3 mm above the adjacent surface of the skin; (+++) raised more than 3 mm above the adjacent surface of the skin.

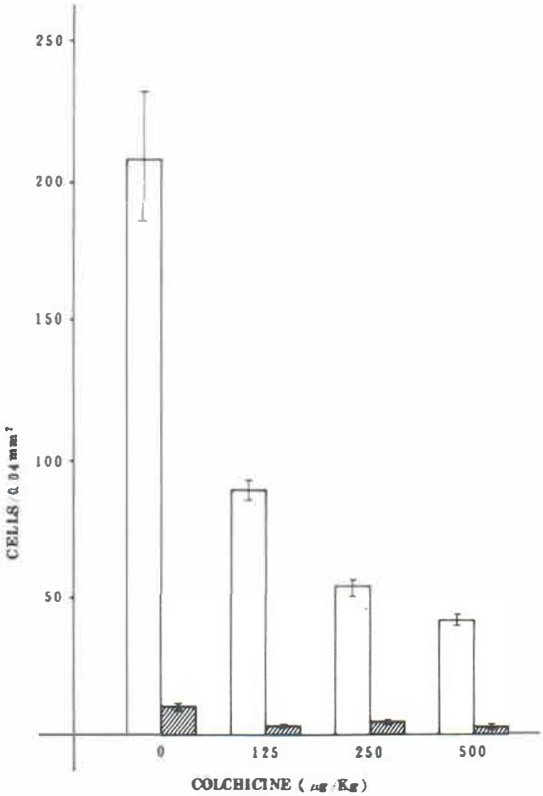

Fig. 2. The number of PMNs (open bars) and MNLs (stippled bars) in the dermal infiltration of DNCB-induced irritant dermatitis at 24 hours.

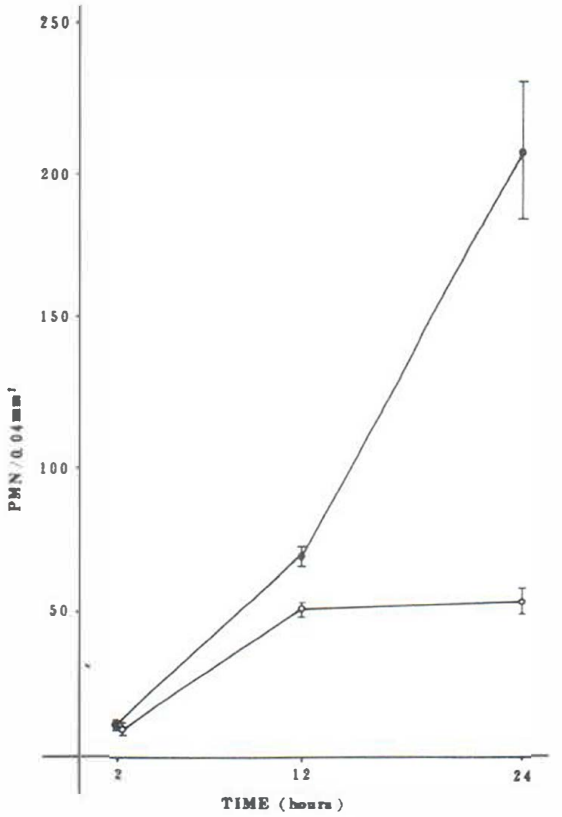

Fig. 3. Time course of dermal infiltrate of PMNs in the lesion of DNCB primary irritant dermatitis with 250 μg/kg of colchicine (open) and saline (closed).

RESULTS

Some of the animals injected with 500 μg/kg of colchicine were exhausted by severe diarrhea. Doses of both 125 and 250 μg/kg caused suppression of DNCB dermatitis macroscopically, histologically and in chemotactic assay, though it was 250 μg/kg that gave the more clear-cut data. This is why we describe chiefly the results induced by 250 μg/kg of colchicine.

Gross skin changes

DNCB-induced erythema was visible both in the colchicine-treated and in the control animals, though its intensity varied from faint to pronounced, depending on the time examined. Scoring of degrees of erythema (Table I) gave no difference in its intensity between the two groups at 2, 12 and 24 hours. On the other hand, induration was reduced by colchicine. In the treated animals, degrees of induration were always one or two grades below those in the control animals.

Histology

The histological changes consisted of epidermodermal separation and dermal infiltrate mainly of PMNs in both the treated and the control animals. Although the reactions reached a maximum at 24 hours in both groups, the intensity of dermal PMN infiltration was reduced by colchicine in a dose-dependent manner (Fig. 1). PMN counting revealed 88.9±4.15 cells/0.04 mm² in the animals treated with 125 μg/kg of colchicine compared with 209±23.02 cells in the control animals. The number of PMNs was reduced when the dose of colchicine was increased (Fig. 2). There was no significant difference in the number of PMNs in the dermal infiltrate at 2 and 12 hours between these two groups (Fig. 3). A few MNLs were seen at 2, 12 and

Table II. *The effect of colchicine on the directional locomotion and the random movement*

	PMNs/10 high-power fields[a] Time (hours)		
	2	12	24
Directional movement			
Colchicine[b] treated (250 μg/kg)	91.0±11.32[c]	95.5±6.69[c]	411.17±21.99[d]
Control[b]	341.0±42.70	477.5±36.32	510.17±58.44
Random movement			
Colchicine[b] treated (250 μg/kg)	37.67±5.31[d]	39.67±4.38[d]	45.33±4.77[d]
Control[b]	44.67±9.07	45.0±4.60	46.67±4.94

[a] Mean ± S.E. [b] $N=6$. [c] $P<0.01$. [d] $P>0.1$

24 hours, and intensity of the infiltration was always the same in both groups (Fig. 2).

Chemotactic assay

The effect of colchicine on directional locomotion and random movement is shown in Table II. Directional locomotion of PMNs induced by LPS-treated serum was markedly affected by colchicine at 2 and 12 hours. However, this effect could no longer be observed at 24 hours, a time when DNCB-produced dermal infiltrate of PMNs was maximum. In contrast, random movement was not influenced by colchicine treatment.

WBC count

The number of peripheral leukocytes and the percentage of PMNs were comparable before and 24 hours after the injection of colchicine.

DISCUSSION

The present study showed that chemotactic activity of PMNs in colchicine-injected animals was maximally inhibited at 12 hours upon *in vitro* Boyden chamber examination and at 24 hours upon in vivo histological examination. We used serum activated by LPS instead of a neutrophil chemotactic factor found in DNCB-induced skin inflammatory lesions as a chemotactic source in *in vitro* examination. Reduced infiltration of PMNs in the inflammatory tissue was preceded by suppressed locomotion of PMNs in the peripheral blood. Hence it was assumed that colchicine reduces the intensity of DNCB-induced irritant dermatitis by affecting the directional movement of PMNs to the skin lesions. It is reported that a transient decrease in the number of PMNs occurs several hours after the colchicine injection (7). Such a decrease might also be responsible for suppression of the inflammation.

It has been demonstrated that the production of PMN chemotactic factor in the DNCB-painted skin peaks between 12 and 24 hours, when infiltration of PMNs in the lesions also reaches a maximum (1). Colchicine treatment suppressed directional movement of PMNs in the peripheral blood, resulting in decreased numbers of migrating PMNs in the lesional skin. One could infer that colchicine inhibited DNCB-dermatitis by reducing the production of chemotactic factor in the lesion. This possibility is unlikely, however, since degrees of damage of the lesional skin were comparable between the two groups, at least after 2 and 12 hours, suggesting that chemotactic factors might be equally produced.

The anti-inflammatory effects of colchicine may be attributed to its ability to bind microtubules (6). Above certain concentrations, colchicine decreases microtubule assembly in neutrophils. Since it is shown that intact microtubules are essential for maximal unidirectional migration during chemotaxis (8), the interference of the microtubular functions of PMN following the treatment with colchicine may result in immobilization of PMN in vivo in the inflammatory lesions. Thus in DNCB-induced irritant dermatitis as well as in other types of inflammation, abolition of microtubules induced by colchicine injection may explain the dysfunction of PMN during the inflammation.

On the other hand, the lack of prominent activity of colchicine in suppressing the carrageenán-induced edema, a MNL-mediated inflammation, as compared with its effect on the reversed passive Arthus reaction, has been noticed. This suggests that colchicine is less efficient in modifying the function of MNL than that of PMN (3). A weak but

comparable infiltration of MNL in both the colchicine-treated groups and the control might be explained on this basis.

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Received October 21, 1980

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