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SHORT REPORTS

Elevated Colchicine Sensitivity of Dermal Lymphocytes in Mycosis Fungoides

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Abstract. Lymphocytes in chronic lymphocytic leukaemia and in other malignant lymphomas exhibit an extreme sensitivity to colchicine and are cytolysed in vitro by 10^{-6} M colchicine. This characteristic can be quantified with the colchicine sensitivity index which is used for diagnostic purposes. We have demonstrated that lymphocytes liberated from skin infiltrates in mycosis fungoides (6 patients) show an elevated colchicine sensitivity of 44 ($p<0.01$); in contrast, the index of lymphocytes isolated from dermal infiltrates of reactive inflammatory dermatoses (9 patients) is low, being only 23. Determination of the colchicine sensitivity index can be helpful in the diagnosis of mycosis fungoides.

Key words: Mycosis fungoides; Dermal infiltrate; Lymphocytes; Colchicine sensitivity

Thomson & Robinson reported in 1967 (8) on the extremely high sensitivity of lymphocytes in chronic lymphocytic leukaemia (CLL) to colchicine. Even 10^{-6} M colchicine concentrations led to a cytolysis of 60–90% of the incubated lymphocytes. To obtain a similar effect on peripheral blood lymphocytes of healthy persons, a concentration 100 000 greater is required (7).

To quantify the action of colchicine on a lymphocyte suspension, a colchicine sensitivity index was introduced (9) and used in the diagnosis of leukaemia (4, 9). Within recent years it has been demonstrated that lymphocytes isolated from lymph nodes in malignant non-Hodgkin lymphomas also exhibit an elevated colchicine sensitivity index, as compared with lymphocytes liberated from reactive lymph node hyperplasia (3).

Investigations into the colchicine sensitivity of lymphocytes in cutaneous malignant lymphomas have not, to our knowledge, been performed until now. The aim of our study was to compare the colchicine sensitivity of lymphocytes isolated from skin infiltrates of various chronic inflammatory dermatoses with those of mycosis fungoides.

METHODS

6 mm punch biopsy samples were taken under local anaesthesia (0.5% Scandicain®) and immediately dissected into small pieces. These were incubated for 15 h at 4°C in 15 ml Eagle's minimal essential medium (MEM), which in addition contained 0.25 M HEPES buffer, 5 mg collagenase (Sigma, London), 0.1 ml 0.5% $CaCl_2$, penicillin (100 I.U./ml), streptomycin (100 µg/ml) and Fungizone® (0.25 µg/ml). Afterwards the lymphocytes were freed from the connective tissue by magnetic stirring (90 min, 37°C). A further liberation of the lymphocytes was accomplished

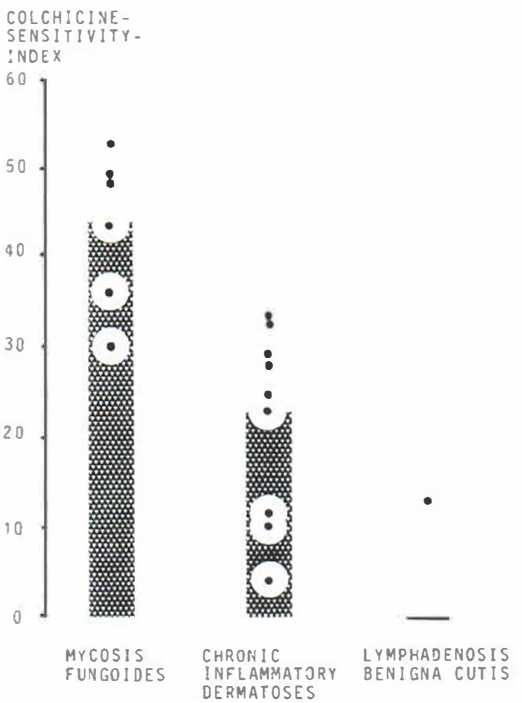

Fig. 1. Colchicine sensitivity index of infiltrating lymphocytes in mycosis fungoides, chronic inflammatory dermatoses and cutaneous pseudolymphoma.

Table I. *Colchicine sensitivity index of infiltrating dermal lymphocytes in mycosis fungoides, chronic inflammatory dermatoses and cutaneous pseudolymphoma*

Patient	Age	Sex		Colchicine sensitivity index	Mean value
R. A.	69	F	Mycosis fungoides, stage II	30	
B. M.	46	M	Mycosis fungoides, stage II	37	
E. I.	36	F	Mycosis fungoides, stage II	49	
W. E.	72	F	Mycosis fungoides, stage II	53	44
U. R.	74	M	Mycosis fungoides, stage II	43	
S. A.	62	F	Mycosis fungoides, stage III	49	
N. N.	30	F	Atopic dermatitis	10	
W. M.	69	M	Atopic dermatitis	4	
M. H.	54	M	Vasculitis	25	
H. A.	79	F	Vasculitis	28	
W. F.	58	M	Prurigo simplex	36	23
S. S.	66	M	Prurigo simplex	35	
W. M.	72	F	Psoriasis	29	
S. O.	31	F	Tuberculin skin reaction	23	
B. H.	35	F	Prurigo nodularis	12	
K. M.	40	F	Lymphadenosis benigna cutis	13	

by aspirating the suspension with a 20 ml plastic syringe and putting it subsequently back into a glass dish for 5 minutes. Then the suspension was filtered through a metal sieve to remove tissue remnants and washed with fresh MEM. The filtered cells were centrifugated (1 500 U/min, 10 min) and again washed with MEM, containing additionally 10% heat-inactivated fetal calf serum (method according to ref. 6).

Cells then were counted in a Neubauer glass chamber and the number of dead cells was determined using the trypan blue exclusion technique. A portion of the suspension was placed on a glass slide and stained with Pappenheim's stain. This ensured that no granulocytes were incubated.

2 ml each of the obtained lymphocyte suspension were incubated with 0.1 and 1.0 μg colchicine/ml. Another 2 ml without colchicine served as control. Incubation was performed at 37°C for 20 h. Afterwards the three specimens were gently resuspended and the number of dead cells was determined with trypan blue exclusion technique.

The effect of colchicine was measured by calculating the percentage of cytolysed lymphocytes, using the formula $100\,(1-A/B)$. A means percentage of surviving lymphocytes after incubation with a certain colchicine concentration; B means the percentage of surviving lymphocytes after incubation without colchicine. The arithmetic mean values of the results from incubation with 0.1 and 1.0 μg colchicine/ml comprised the colchicine sensitivity index.

MATERIAL

15 patients were included in the study. Six suffered from histologically proved mycosis fungoides. The other 9 patients had chronic inflammatory benign skin diseases [prurigo simplex: 2; atopic dermatitis: 2; vasculitis: 2; prurigo nodularis: 1; proriasis: 1; tuberculin skin reaction: 1]. Additionally, the colchicine sensitivity index of one patient with lymphadenosis benigna cutis was determined.

RESULTS

The colchicine sensitivity index in mycosis fungoides is nearly twice as high as in chronic inflammatory dermatoses (Fig. 1, Table I).

Lymphocytes isolated from cutaneous infiltrates of mycosis fungoides showed a mean index of 44, the individual values ranging between 30 and 53. In contrast, lymphocytes liberated from benign chronic dermal inflammations had a mean index of 23.

The difference between these groups is highly significant ($p<0.01$, Wilcoxon test).

Lymphocytes isolated from one patient with lymphadenosis benigna cutis showed an index of 13 (Fig. 1, Table I).

DISCUSSION

Colchicine, an alkaloid from meadow saffron (*Colchicum autumnale*) known from ancient times, exerts a potent inhibition on mitosis during metaphase. The underlying mechanism is the destruction of the microtubules in the cell spindle (7). Concerning the cytolysis of lymphocytes from malignant lymphomas (CLL, diffuse and nodular non-Hodgkin lymphomas), colchicine acts on cells in the

interphase. The action site of this effect is again the microtubules, as recent investigations have shown (7). Microtubules are built up of a protein polymer, which is synthesized during the interphase, and can be demonstrated by electron microscopy. Colchicine reacts with the monomers of the microtubule protein, which are in equilibrium with the polymer; ultrastructurally no microtubules are demonstrable (7). The uptake of ^{3}H-labelled colchicine into lymphocytes, however, does not reveal any difference between CLL lymphocytes and normal lymphocytes (7).

Besides the destruction of microtubules, CLL lymphocytes exhibit damaged mitochondriae and plasma membrane following colchicine incubation (7).

The colchicine sensitivity index of lymphocytes from chronic reactive inflammatory dermatoses is most frequently found between 20 and 30, whereas the index of lymphocytes from reactive lymph node hyperplasia never exeeds 20 (3). This is probably due to the greater damage during isolation of the lymphocytes from skin connective tissue. At present we are trying to develop techniques for a more rapid but gentler isolation of lymphocytes from dermal infiltrates.

The colchicine sensitivity index is a parameter which should be determined in special cases for judgement of the dignity of a dermal infiltrate; this is also illustrated by the low index in cutaneous pseudolymphoma (lymphadenosis benigna cutis). Further studies will show whether the colchicine sensitivity index can be used to check therapeutic measures or to elicit information on the prognostic characteristics in cutaneous malignant lymphomas.

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Pityriasis Lichenoides, an Immune Complex Disease?

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Abstract. Nine biopsies from skin lesions of 5 patients with pityriasis lichenoides acuta and three biopsies from skin lesions of 3 patients with pityriasis lichenoides chronica were examined by means of the direct immunofluorescence technique. IgM deposits along the dermo-epidermal junction were found in only two biopsies. In the majority of biopsies, complement (C_3) deposits were found along the dermo-epidermal junction and in the vessel walls. Immunoglobulin and C_3 deposits were not found concomitantly in the vessel walls.

Key words: Pityriasis lichenoides; Immunofluorescence technique

Data on immunofluorescence studies in patients with pityriasis lichenoides (PL) are still sparse, and are rather contradictory.

Recently, Clayton et al. (2) and Clayton & Haffenden (3) described the presence of IgM and C_3 along the dermal–epidermal junction (DEJ) of skin lesions and the presence of immune complexes in the circulation of patients with PL. On the basis of these findings the authors propose to consider PL as an immune complex disease.