THE EFFECT OF LONG-TERM COLCHICINE THERAPY IN PATIENTS WITH RECURRENT POLYSEROSITIS ON THE CAPACITY OF BLOOD PLATELETS TO SYNTHESIZE THROMBOXANE A₂

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Thromboxane B₂ (TXB) production during platelet activation was measured in 13 patients with recurrent polyserositis on long-term colchicine therapy and 10 healthy controls. Plasma colchicine concentration in patients ranged between 0.8–7.8 ng/ml ($2.0\text{--}19.5 \times 10^{-9}$ M). Thromboxane production induced during aggregation by arachidonic acid or collagen and serum TXB₂ levels (clotted blood, 37°C, 1 h) did not differ among patients and controls. Previous studies reporting platelet function defects including anomalous TXB₂ production, were performed *in vitro* using colchicine concentrations markedly exceeding the therapeutic range.

Keywords colchicine thromboxane A₂ recurrent polyserositis

Introduction

Colchicine is a veteran drug used for centuries for the treatment of acute gouty arthritis. More recently, it has been effectively employed for the prophylaxis of acute attacks of recurrent polyserositis (RP, familial Mediterranean fever) (Eliakim *et al.*, 1981) as well as in Behçet's disease (Mirushima & Matsumura, 1979). Unlike other anti-inflammatory agents which exert their activity through the inhibition of prostaglandin synthesis, colchicine has been found to stimulate the production of prostaglandin E and the synthesis of thromboxane A₂ in experimental models (Robinson 1975; Floman, 1978; Horrobin *et al.*, 1978). Significant amounts of thromboxane A₂ are produced during platelet aggregation, depending on the agents used to stimulate the platelets. Colchicine affects platelets both structurally and functionally. It has been reported to cause disassembly of microtubules and inhibition of the second phase of aggregation (Gold *et al.*, 1973; White, 1969a,b; Bibbi & Apitz-Castro, 1975; Menache *et al.*, 1980). *In vitro* studies of the effect of colchicine on prostaglandin synthesis by human platelets are inconclusive as both an increase in malondialdehyde (MDA) formation (Martin & Greaves, 1982), and a decrease in TXB₂, with little or no effect on PGE₂ have been reported (Horrobin *et*

al., 1978). These differences may relate to the concentration of colchicine employed.

We report that in recurrent polyserositis patients on long-term effective colchicine therapy, the capacity of the platelets to synthesize TXA₂ was unchanged.

Methods

Thirteen patients receiving long-term colchicine treatment for the prophylaxis of acute attacks of recurrent polyserositis (FMF) were examined (Table 1). Nine were male and four were female. Their ages ranged from 12–55 years. They had all experienced typical clinical manifestations of recurrent polyserositis (Eliakim *et al.*, 1981) which responded to colchicine therapy. Colchicine was given orally in one or two daily doses, the total daily dosage ranging from 1.0–2.0 mg. The mean duration of therapy was 4.7 years.

The patients were being followed at the outpatient clinic of the Department of Medicine A, Hadassah University Hospital, Jerusalem, at 3 monthly intervals. All of them consented to have 20 ml of blood

Table 1 Patient characteristics. Colchicine therapy and plasma colchicine levels in patients with recurrent polyserositis.

Patient	Sex	Age (years)	Colchicine dose (mg/day)	Duration of therapy (years)	Plasma colchicine concentration (ng/ml)	($M \times 10^{-9}$)
1	M	20	1.0	6	3.2	8.0
2	M	20	2.0	4	4.8	12.0
3	M	12	1.0	2	2.1	5.3
4	M	20	1.0	1	5.2	13.0
5	M	22	2.0	5	3.2	8.0
6	M	44	1.0	4	1.7	4.3
7	F	55	1.0	6	0.9	2.3
8	M	22	1.0	8	1.0	2.5
9	M	43	1.0	8	0.8	2.0
10	F	42	1.0	6	3.8	9.5
11	F	26	1.0	1	3.6	9.0
12	M	20	1.0	5	2.7	6.8
13	F	18	2.0	6	7.8	19.5

drawn for a complete blood count, platelet function tests and the determination of plasma colchicine concentration. Samples were drawn from 2–16 h after the last colchicine administration. Ten healthy volunteers, medical technicians and students, five males and five females, aged 25–38 years served as controls. Neither the patients nor the volunteers received other drugs in the 2 preceding weeks. Complete blood counts were performed with a Coulter Counter Model S+Plus.

Platelet aggregation

Venous blood was mixed with 0.1 vol. of 3.2% trisodium citrate. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared as previously reported (Eldor *et al.*, 1983). Platelet aggregation studies were performed in a dual channel Payton aggregation module (Payton Associates, Buffalo, New York, USA) as previously reported (Eldor *et al.*, 1983). Two aggregating agents were used, arachidonic acid (as the sodium salt in 0.1 M Na_2CO_3 (Sigma Chemical Co., St Louis, Missouri, USA) final concentration 0.3 μM) and collagen (Hormon-Chemie, München, Federal Republic of Germany) in a final concentration of 0.8 $\mu\text{g/ml}$). The final reaction volume was 0.5 ml. The extent of aggregation was determined by the percentage of the largest change in light transmission within 4 min after the addition of the stimulus of the full range between PRP and PPP. All aggregation studies were performed in duplicate.

Thromboxane A_2 (TXA_2) release

Samples (0.1 ml) were withdrawn from the aggregation cuvette 4 min after the addition of the aggregation agent. Samples were quenched with 0.9 ml 0.1 M

phosphate-buffered saline containing 10^{-4} M indomethacin (Sigma Chemical Co.), frozen in liquid N_2 and stored in -70°C until assayed for TXB_2 by a radioimmunoassay.

Thromboxane A_2 synthesis during blood clotting

Samples (1.0 ml) were incubated in glass tubes at 37°C for 1 h immediately after blood drawing. At the end of the incubation, the samples were centrifuged in Eppendorf 5212 for 1 min, serum was withdrawn and stored in -70°C until assayed for TXB_2 . Tests were performed in triplicate.

Radioimmunoassays for TXB_2

Specific antiserum to TXB_2 was a kind gift from Dr B.B. Weksler, Cornell Medical College, New York. The specificity and cross-reactivity of the antibody has previously been described (Levin *et al.*, 1981). Radioimmunoassay for TXB_2 was performed according to the method of Bauminger *et al.* (1973).

Plasma concentration of colchicine

Plasma colchicine levels were determined by a radioimmunoassay developed in our laboratory. Antiserum to colchicine was prepared by the method of Boudene *et al.* (1975). Plasma samples (0.2–0.4 ml) were prepared from heparinized blood and incubated with 0.1 ml of the appropriate antiserum dilution (to give about 50% binding) and 0.1 ml (15,000 counts/min ca 0.64 ng) of [^3H]-colchicine (specific activity 10.1 Ci/mmol, New England Nuclear) in a buffer solution containing 0.01 M sodium phosphate, pH 7.4, 0.15 M sodium chloride, 0.1% bovine serum albumin, and 0.1% bovine serum albumin, and 0.1%

sodium azide. The volume of the incubation mixture was adjusted to 1.0 ml. After incubation for 20 h at 4°C in the dark, unbound colchicine was absorbed on dextran-coated charcoal (0.2 ml of a 1.25% Norit A neutral charcoal, 0.125% Dextran T-70 in buffer) and the percent bound was determined by liquid scintillation spectrometry (Biogluor, New England Nuclear). The minimal detectable concentration of colchicine was 0.3 ng/ml.

Statistical method

The correlation between the plasma concentrations of colchicine and the effect of various platelet function parameters was determined by the least square linear regression analysis. Statistical significance was tested by the Student's *t*-test.

Results

Peripheral blood counts

Blood counts were normal in all patients, except in patient number 10 who had iron deficiency anaemia.

Haemoglobin levels ranged from 10.3–16.9 g/100 ml (mean $\pm$ s.e. mean 14.0 ± 0.5), the leukocyte counts from 5.4 – $11.5 \times 10^9/l$ (mean $\pm$ s.e. mean 7.5 ± 0.48) and the platelet counts from 1.87 to $4.07 \times 10^{11/l}$ (mean $\pm$ s.e. mean 2.88 ± 0.17).

Plasma colchicine levels

Measurable levels of colchicine were present in all patient plasma samples (Table 1). These ranged between 0.8–7.8 ng/ml (2.0 – 19.5×10^{-9} M). No correlation was looked for between the dose of colchicine and the plasma levels, as timing between the last drug administration and blood drawing varied.

Thromboxane production

TXB₂ production was measured during platelet aggregation induced by arachidonic acid and collagen as well as in serum prepared from blood which had been incubated as 37°C for 1 h. The extent of aggregation with both agents was similar in platelets obtained from patients on long-term colchicine ther-

Table 2 Platelet aggregation and TXB₂ formation in recurrent polyserositis patients treated with colchicine.

Number	Arachidonic acid (3 μ M)		Collagen (0.8 μ g/ml)		TXB ₂ in (clotted blood (ng/ml) (1 ml, 37°C, 1 h))
	aggregation*	TXB ₂ (ng/ml)	aggregation	TXB ₂ (ng/ml)	
1	65	378	64	27.7	128
2	68	367	69	21.9	200
3	67	198	50.5	20.8	—
4	75	219	63	20.4	—
5	66	319	63	22.9	222
6	73	309	—	—	530
7	68	353	73	53.7	377
8	73	240	72	36.7	341
9	76	330	74	23	209
10	73	472	69	47	137
11	74.5	712	73	33.5	213
12	71	325	70	20	62.8
13	80	240	75	15	102
Mean	71.5	343.2	67.9	28.6	229.3
s.e. mean	1.2	35.7	1.9		39.5
Controls					
1	70	618	70	45.5	684
2	77	335	73	26	261
3	76	635	74	35.5	180
4	74	442	63.5	31	337
5	75	360	73	45.5	114
6	76	427	77	16.2	458
7	77	119	71	14.5	180
8	61	297	58	21.7	258
9	68	255	60	21.9	365
10	63	147	63	15.9	85
Mean	71.7	363.5	68.3	27.4	292.2
s.e. mean	1.8	52.2	1.9	3.5	53.9

* % aggregation as indicated in materials and methods.

apy and non-medicated healthy subjects (Table 2). The levels of thromboxane B₂ during aggregation and in serum samples from patients and controls did not differ significantly (Table 2). There was no correlation between the extent of aggregation or thromboxane production induced by the two agonists, and the plasma colchicine concentration. The same is true for thromboxane production in clotted blood.

Discussion

Several studies using both rat synovium and human platelets, have shown that colchicine increased prostaglandin formation (Robinson, 1975; Floman, 1978; Horrobin *et al.*, 1978; Martin & Greaves, 1982). However, this phenomenon was observed with colchicine concentrations which were several orders of magnitude above toxic concentration. Different results, namely, inhibition of TXB₂ formation by platelets, were observed *in vitro* with concentrations of 10^{-8} – 10^{-7} M (Horrobin *et al.*, 1978). The *in vivo* effects of prolonged colchicine therapy on prostaglandin formation in humans has so far not been tested. From the data presented herein, it is evident that the capacity of platelets to produce TXB₂ is not impaired in patients treated effectively for years with

colchicine, and in whom the presence of the drug was demonstrated in the plasma. TXB₂ formation was measured during platelet activation, stimulated with two agonists: arachidonic acid and collagen. With both agents TXB₂ production was similar in the patients' platelets and the non-medicated healthy subjects. Furthermore, in another test, in which platelet activation is induced by thrombin generated during clotting of the blood (Patrino, 1980), the amounts of TXB₂ formed were unaltered in treated patients. The differences in results between the *in vitro* studies and the present *in vivo* study may well be due to differences in colchicine concentrations, which in our patients do not exceed 2×10^{-8} M (Zylber-Katz *et al.*, 1982).

Clinical experience shows that patients with recurrent polyserositis do not exhibit any haemorrhagic or thrombotic tendencies (Eliakim *et al.*, 1981; Heller *et al.*, 1958). Our experience in more than 170 recurrent polyserositis patients on long-term colchicine therapy also did not reveal any clinical manifestations associated with the haemostatic system.

The technical assistance of Ms Susan Jackson is gratefully acknowledged. This work was supported in part by a grant from the A. Jurzykowski Foundation, New York.

References

- BAUMINGER, S., ZOR, Y. & LINDER, H.R. (1973). Radio-immunological assay of prostaglandin synthetase activity. *Prostaglandins*, **4**, 313–324.
- BIBBI JAFFE, A. & APITZ-CASTRO, R. (1975). The effect of colchicine on human blood platelets under conditions of short term incubation. *Biochem. J.*, **178**, 449–454.
- BOUDENE, C., DUPREY, F. & BOHOUN, C. (1975). Radio-immunoassay of colchicine. *Biochem. J.*, **151**, 413–415.
- ELDOR, A., POLLIACK, G., VLODAVSKY, A. & LEVY, M. (1983). Effects of dipyrone on prostaglandin production by human platelets and cultured bovine aortic endothelial cells. *Thromb. Hemostas.* (in press).
- ELIAKIM, M., LEVY, M. & EHRENFELD, M. (1981). *Recurrent polyserositis (Familial Mediterranean Fever, Periodic Disease)*. New York: Elsevier North Holland Inc.
- FLOMAN, Y. (1978). Prostaglandin E production by cultured inflamed rat synovium: Stimulation by colchicine and inhibition by chloroquine. *Prostaglandins Med.*, **1**, 39–48.
- GOLD, M. (1973). Platelet factor 3 activity made available from human platelets by ADP. Inhibition by colchicine. *Thrombos. Diathes. Haemorrh. (Stuttg.)*, **30**, 155–159.
- HELLER, H., ZOCHAR, E. & SHERF, L. (1958). Familial Mediterranean fever. *Arch. Intern. Med.*, **102**, 50–71.
- HORROBIN, D.F., OKA, M. & MANKU, M.S. (1978). Thromboxane A₂ as a regulator of prostaglandin biosynthesis. Relevance to interactions between colchicine and histidine in control of inflammation. *Eur. J. Rheum.*, **1**, 181.
- LEVIN, R.I. (1981). Nitroglycerin stimulates synthesis of prostacyclin by cultured human endothelial cells. *J. clin. Invest.*, **67**, 762–763.
- MARTIN, J.F. & GREAVES, M. (1982). Vincristine inhibits the synthesis of malondialdehyde by human platelets *in vitro*. *Cancer*, **49**, 665–668.
- MENACHE, D., ISRAEL, A. & KARPTAKIN, S. (1980). Platelet and microtubules: Effect of colchicine and D₂O on platelet aggregation and release induced by calcium ionophase A23187. *J. clin. Invest.*, **66**, 289–291.
- MIRUSHIMA, Y. & MATSUMURA, M. (1979). Chemotaxis of leukocytes and colchicine treatment in Behcet's disease. *J. Rheumatol.*, **6**, 108–110.
- PATRINO, C. (1980). Radioimmunoassay of serum thromboxane B₂ a simple method of assessing pharmacologic effects on platelet function. In *Advances in prostaglandin and thromboxane research*, Vol. 6. ed. Samuelsson, B., Ramwell, P.W. & Paoletti, R., pp. 187–191. New York: Raven Press.
- ROBINSON, D.R. (1975). Prostaglandin synthesis by rheumatoid synovia and its stimulation by colchicine. *Prostaglandins*, **10**, 67–85.
- WHITE, J.G. (1969a). Effects of colchicine and vinca alkaloids on human platelets: I. Influence on platelet microtubules and contractile function. *Am. J. Path.*, **53**, 281–291.
- WHITE, J.G. (1969b). Effects of colchicine and vinca alkaloids on human platelets: III. Influence on primary internal contraction and secondary aggregation. *Am. J. Path.*, **54**, 467–478.
- ZYLBER-KATZ, E. (1982). Plasma colchicine concentrations in patients with recurrent polyserositis (Familial Mediterranean Fever) on long term prophylaxis. *Arth. Rheum.*, **25**, 227–231.

(Received January 10, 1983,
accepted April 19, 1983)