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Colchicine derivatives inhibit neopterin production in human peripheral blood mononuclear cells (PBMC)

Z. Z. ALTINDAG*, G. WERNER-FELMAYER, G. SAHIN*, H. WACHTER & D. FUCHS *Institute for Medical Chemistry and Biochemistry, University of Innsbruck and Ludwig Boltzmann Institute of AIDS Research, Innsbruck, Austria, and *Department of Pharmaceutical Toxicology, Faculty of Pharmacy, University of Hacettepe, Ankara, Turkey*

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SUMMARY

Colchicine is a microtubule disrupting agent, mostly used as treatment in various kinds of inflammatory diseases such as acute familial Mediterranean fever and Behcet's disease, as well as gout. In patients with familial Mediterranean fever treatment with colchicine induces a decline of urinary neopterin concentrations which indicates a decrease of cell-mediated immune activation. In this study, we investigated a potential effect of colchicine on the T cell/macrophage system *in vitro*. The human myelomonocytic cell line THP-1 and PBMC were treated with colchicine or the colchicine derivative, colcemide, in the presence or absence of 250 U/ml interferon-gamma (IFN-γ) or 10 μg/ml lipopolysaccharide (LPS) for 48 h or 96 h. Colchicine and colcemide increased neopterin/protein production in unstimulated THP-1 cells, but no such effect was apparent in cells stimulated with IFN-γ. By contrast, when PBMC were treated with colchicine or colcemide a significant reduction in neopterin formation was evident in cells without and with prestimulation by IFN-γ or LPS. In parallel, reduced production of IFN-γ was observed in PBMC. These data suggest that colchicine and colcemide are able to inhibit T cell activation within the cellular immune response.

Keywords colchicine colcemide neopterin cellular immunity THP-1 cells peripheral blood mononuclear cells interferon-gamma

INTRODUCTION

Colchicine, known as an anti-inflammatory drug, binds at the R site on β-tubulin and causes microtubule depolymerization [1]. In this way it stops cell division in metaphase by inhibiting the movement of chromosomes within microtubules, and so can interfere with various intracellular pathways as well as secretory processes [2]. As colchicine suppresses neutrophil chemotaxis and migration of granulocytes into inflamed areas [3], it is often used for therapy of inflammatory diseases such as Behcet's disease [4], gout [3] and acute familial Mediterranean fever (FMF), in order to decrease the frequency of attacks and to prevent the development of amyloidosis in the latter condition [5–7].

Neopterin is a metabolite of guanosine triphosphate, and large amounts of it are secreted by human monocytes/macrophages upon activation by T cell-derived interferon-gamma (IFN-γ) *in vitro* [8,9]. Raised neopterin levels are commonly observed in body fluids in patients with diseases involving cellular immunity, such as allograft rejection, autoimmune diseases, infections and certain malignant disorders [10–14]. Recently, increased urinary neopterin excretion was described in patients with FMF during acute attacks, and neopterin levels declined rapidly when patients were treated with colchicine [15]. This observation prompted us to investigate whether colchicine had any direct influence on effector cells of the cellular immune system. For this purpose we investigated the influence of colchicine and of its close analogue, colcemide, on neopterin production by the human myelomonocytic cell line THP-1 and by PBMC.

Correspondence: Dr Dietmar Fuchs, Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Fritz-Pregl Str. 3, A-6020 Innnsbruck, Austria.

MATERIALS AND METHODS

Cell culture

Buffy coat cells from blood of healthy donors were kindly provided by the Blood Transfusion Centre University Hospital, Innsbruck. PBMC were enriched from buffy coats by a two-step density-gradient centrifugation over Ficoll–Paque. Cells were seeded in 10 ml RPMI 1640 medium (endotoxin concentration <1 pg/ml) with 10% (v/v) heat-inactivated fetal calf serum (FCS; Biochrom, Berlin, Germany), 2 mM L-glutamine, 100 U/ml penicillin and 0·1 ng/ml streptomycin, at a density of $2{\cdot}4 \times 10^6$/ml in 24-well plates (Falcon, Becton Dickinson, Plymouth, UK). Solutions of colchicine and colcemide in PBS (Serva, Feinbiochimica, Heidelberg, Germany) were added to the PBMC to give

concentrations ranging from 10 ng/ml to 1 μg/ml in the presence or absence of 250 U/ml recombinant human IFN-γ (specific activity 2×10^7 U/mg; Bioferon, Laupheim, Germany), or 10 μg/ml lipopolysaccharide (LPS; phenolic extract from *Escherichia coli* 055 : B5; Sigma, Munich, Germany) dissolved in RPMI medium. Cells were incubated for 48 h and 96 h at 37°C in humidified air containing 5% CO_2. At the end of the incubation period, cells were harvested, centrifuged at 400 ***g*** and the supernatants were frozen at −20°C or immediately used for neopterin and IFN-γ determination.

THP-1 cells, a human myelomonocytic cell line, were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in RPMI 1640 medium (see above) and seeded at a density of 10^6 cells/ml in 24-well plates. Colchicine and colcemide (see above) alone or together with 250 U/ml IFN-γ were administered to the cells. The cells were incubated for 48 h at 37°C in the same conditions as described above for PBMC. At the end of the incubation period cells were harvested, centrifuged at 400 ***g*** and the supernatants were taken immediately and frozen at −20°C.

Determination of neopterin and IFN-γ

Neopterin was determined by a commercially available enzyme immunoassay (ELItest Neopterin; BRAHMS/Henning, Berlin, Germany) according to the manufacturer's instructions. The results are expressed as pmol/mg protein. IFN-γ production in PBMC was determined by an enzyme immunoassay for quantitative measurement (Interferon gamma ELISA; Endogen Inc, Cambridge, MA). Accuracy and specificity of both tests for measurements of cell culture supernatants were proved.

Protein determination

Protein contents were estimated with the BioRad protein assay according to Bradford, using freeze-dried pure bovine serum albumin (BSA) as standard [16].

Statistical analysis

Results were expressed as mean ± s.e.m. Statistical evaluation was done using Student's *t*-test.

RESULTS

Treatment of THP-1 cells with colchicine or colcemide had an inconsistent effect on neopterin production. In unstimulated cells no change of absolute neopterin concentrations was seen after treatment with colchicum derivatives, with the exception of an increase induced with 100 ng/ml colcemide. When neopterin concentrations were expressed per mg protein, colchicine and colcemide caused an increase of neopterin release (Table 1). Divergent results were seen in IFN-γ-treated THP-1 cells. A decrease of neopterin production was induced by both colchicum derivatives, higher concentrations leading to greater suppression of neopterin release. However, neopterin per protein content remained stable, with the exception of 10 ng/ml colcemide when the decrease remained significant (Table 1).

In PBMC, colchicine and colcemide were able to reduce neopterin production. After incubation of cells for 48 h a decrease of neopterin production was observed from cells stimulated with LPS or IFN-γ, but in unstimulated controls also baseline neopterin production was suppressed (Fig. 1). The effects of colchicine and colcemide were more pronounced in cells when incubation was extended to 96 h (Fig. 1). There were no apparent morphological abnormalities of cells seen even at the highest concentrations of colchicine and colcemide (1000 ng/ml) and no cytotoxicity was observed (Celltiter 96 Aqueous Non-Radioactive Cell Proliferation Assay; Promega, Madison, WI).

In a similar way, IFN-γ production in cells was suppressed by the colchicum derivatives. IFN-γ in supernatants was reduced in the presence and in the absence of LPS following incubation for 48 h (Table 2). Even the lowest concentration of colchicine was able to suppress spontaneous IFN-γ production by PBMC. There was no influence of the compounds on protein concentrations in these cells.

DISCUSSION

When T cells are activated, several immunomodulatory cytokines such as IL-2, IL-4 and IFN-γ are released [17]. IFN-γ is the most important activator of macrophages to induce the production of various cytotoxic components, e.g. reactive oxygen intermediates

Table 1. Neopterin concentrations in supernatants from THP-1 cells treated with colchicine or colcemide in the presence or absence of rhu-IFN-γ for 48 h

	Neopterin (nmol/*l*)		Neopterin (pmol/mg protein)	
Treatment	None	250 U/ml IFN-γ	None	250 U/ml IFN-γ
None	2·06 ± 0.13	31·3 ± 0·86	70·4 ± 4·19	1118 ± 51·9
Colchicine (ng/ml)				
10	2·25 ± 0·12	27·2 ± 1·40*	83·6 ± 4·67	1088 ± 34·3
100	2·06 ± 0·10	22·1 ± 1·45***	96·9 ± 5·00***	1148 ± 42·6
1000	1·94 ± 0·10	17·0 ± 0·83***	93·1 ± 5·15***	1129 ± 46·6
Colcemide (ng/ml)				
10	2·48 ± 0·30	25·9 ± 0·91**	64·1 ± 4·44	888 ± 26·7***
100	2·74 ± 0·20*	24·7 ± 1·03**	92·7 ± 4·95**	1181 ± 40·3
1000	2·02 ± 0·16	17·0 ± 0·73**	84·2 ± 5·34*	1138 ± 58·9

Results (mean ± s.e.m.) are representative of three different experiments run in triplicates. *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$.

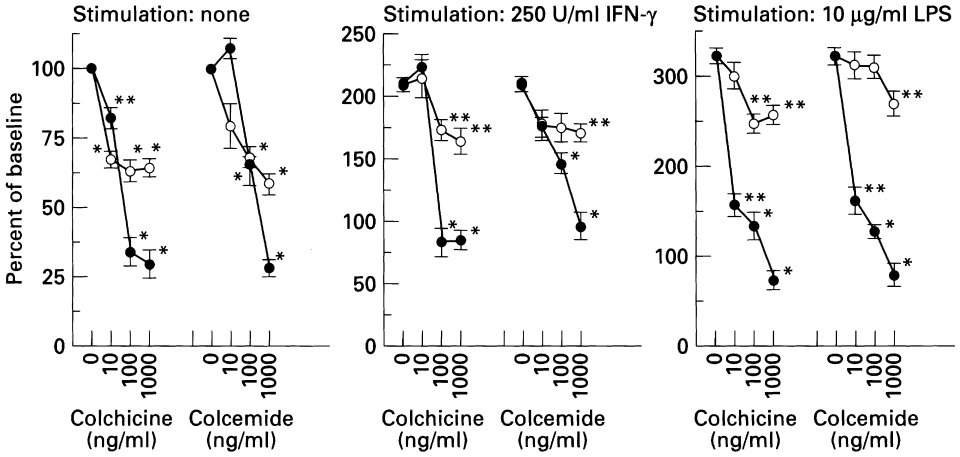

Fig. 1. Neopterin in percentage of baseline (untreated cells at 48 h, 261 ± 16 pmol/mg protein; and 96 h, 1596 ± 63 pmol/mg protein, respectively) in supernatants from PBMC following treatment with colchicine or colcemide after incubation for 48 h (○) and 96 h (●). Results represent mean values ± s.e.m. of three different experiments run in triplicates. $^{**}P < 0{\cdot}05$; $^{*}P < 0{\cdot}01$.

[18], and it also induces a number of cytokines which further contribute to maintenance of the immunoregulatory cascade [17,19,20]. In turn, IFN-γ stimulates neopterin production in human monocytes/macrophages, and the neopterin concentration in body fluids is a sensitive indicator of the activation of cell-mediated immunity [9,10].

The present study has demonstrated an inhibitory effect of colchicine and colcemide on neopterin production and release in PBMC. Colchicine or colcemide also decreased absolute concentrations of neopterin in THP-1 cells stimulated with IFN-γ, but in this case the effect disappeared almost completely if concentrations were related to protein contents. Moreover, colchicum derivatives were even capable of increasing neopterin/protein concentrations in unstimulated THP-1 cells. Similarly, Ferrua *et al.* have found that these drugs can induce production of IL-1β in unstimulated THP-1 cells [21]. Notably, no correction for protein content was calculated by Ferrua *et al.*, and the concentrations applied were higher than those in our experiments, but in general these and our results support the notion that there exists some stimulatory capacity of colchicum derivatives on resting monocytic cells.

Table 2. Production of IFN-γ by PBMC treated with colchicine or colcemide in the presence or absence of 10 μg/ml lipopolysaccharide (LPS) for 48 h

ng/ml	None pg/mg protein	Stimulated with LPS pg/mg protein
None	20·7 ± 1·0	39·5 ± 5·3
Colchicine		
10	6·9 ± 0·7**	32·9 ± 1·8
100	6·3 ± 0·7**	8·9 ± 0·9*
1000	5·2 ± 0·3***	6·7 ± 0·3*
Colcemide		
1000	4·3 ± 0·4***	5·3 ± 0·2*

Values are expressed as mean ± s.e.m. ($n = 3$). $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

There was a decline in neopterin production when PBMC were treated with colchicum derivatives, even in the absence of prestimulation of cells by IFN-γ or LPS. Because IFN-γ production in PBMC was also suppressed by colchicine and colcemide, the data suggest that the drugs can inhibit T cell activation and that the effects of those compounds on neopterin production by PBMC are merely due to their interaction with T cells rather than with monocytes/macrophages. It is well established that neopterin secretion is directly related to the exposure to IFN-γ [8,9,22,23], and a suppression of IFN-γ production by PBMC would in turn decrease neopterin production. In our study, the total effect of colchicum derivatives on the mixed population of PBMC is suppressive with respect to neopterin production rates.

Thus far, monocytes/macrophages are considered to be the sole relevant source of neopterin formation [10]. Earlier we have demonstrated that the human myelomonocytic cell line THP-1 behaves similarly to human monocytes/macrophages isolated from peripheral blood with respect to the conditions necessary for neopterin production [22]. Certainly the tumour cell line THP-1 cannot be regarded as functionally equivalent to mature peripheral blood monocytes, but the inhibitory capacity of colchicum derivatives on IFN-γ production by PBMC seems much more drastic than their eventual stimulatory effect on monocytic cells.

When PBMC were stimulated with LPS or IFN-γ for 48 h and treated with colchicine or colcemide, there was a decrease in neopterin production, and the effect was much more pronounced when cells were stimulated for 96 h. The data agree with reduced neopterin levels observed in FMF patients under treatment with colchicine [15], and may indicate that these agents interfere with the activation of T lymphocytes and reduce production of IFN-γ also in the *in vivo* situation. This view is supported by earlier data obtained from mixed lymphocyte culture experiments in rats, in which a significant reduction of lymphocytic proliferation and enhanced survival of renal allografts were seen during long-term use of colchicine [24]. Also in humans colchicine was found to be able to inhibit cytokine production.

Colchicine was found to reduce the increased production of IL-1 in patients with primary biliary cirrhosis as well as to inhibit fibroblast proliferation [25]. It has to be kept in mind that the concentrations used for treatment are much lower than those tested

in the *in vitro* experiments, but treatment of FMF patients with colchicine resulted in decreasing suppressor T cell counts and increasing natural killer (NK) cell numbers, and it reduced IL-2 production [26].

We conclude that colchicine and its analogue colcemide inhibit IFN-γ and neopterin production in PBMC. A role for colchicine in the regulation of the immune system seems to be evident.

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