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Mechanism of the anti-inflammatory effect of colchicine in rheumatic diseases: a possible new outlook through microarray analysis

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Objective. **Colchicine is an alkaloid that is used to alleviate acute gout and to prevent acute attacks of familial Mediterranean fever (FMF). However, it is not beneficial when given during the occurrence of an acute episode of FMF. It is believed that colchicine exerts its anti-inflammatory effect through direct interaction with microtubules. We aim to study the molecular basis of colchicine action by analysing the effect of this drug on global gene expression of HUVEC (human umbilical vein endothelial cell line) cells.**

Methods. **HUVEC cells were exposed to various concentrations of colchicine and were harvested at different time points. Ribonucleic acid was extracted, amplified, reverse transcribed and hybridized to complementary deoxyribonucleic acid microarrrays containing more than 40,000 probes to human expressed sequence tags. This approach enabled us to have a global look at the transcriptional response induced by colchicine treatment.**

Results. **Colchicine changed the expression of many genes in HUVEC cells following exposure to a concentration of 100 ng/ml or higher. Following short exposure (30 or 120 min), colchicine affected genes known to be involved in the cell cycle and its regulation. However, change in expression of genes involved in neutrophil migration or other inflammatory processes were observed mainly after 12 to 24 h.**

Conclusions. **The anti-inflammatory effect of colchicine may be mediated not only through direct interaction with microtubules but also through changes at the transcriptional level. This latter effect apparently requires a higher concentration and a longer time to occur. This can explain the observation that colchicine does not have an immediate effect when given during an acute attack of FMF.**

KEY WORDS: Colchicine, Familial Mediterranean fever, HUVEC cells, Anti-inflammatory.

Colchicine is an alkaloid that has been used for centuries in acute gouty arthritis. In the last 50 yr it has been employed for an increasing number of diseases including familial Mediterranean fever (FMF), Behçet's syndrome (BS), Sweet's syndrome, scleroderma, amyloidosis and liver cirrhosis [1].

In acute gout, colchicine is effective in alleviating the acute attack and as a prophylactic medication. In Behçet's disease, colchicine is effective mainly in the treatment of erythema nodosum, arthritis and mucosal ulcers, especially in female genitalia [2]. In scleroderma, it may decrease the stiffness of the skin, whereas in amyloidosis it may improve proteinuria and result in regression of amyloid deposition in cases where these fibres are deposited [3]. Perhaps the most effective results of colchicine treatment have been obtained in the prophylaxis of FMF [4]. In this disease, it prevents the occurrence of the acute inflammatory episodes and fends off the development of amyloidosis. However, it is not effective in controlling acute attacks when administered while they occur.

Colchicine consists of three hexameric rings termed A, B and C. The tropolone methyl ester, which is a precise analogue of the ring C of colchicine, can bind the tubulin molecule thereby inhibiting its polymerization into microtubules *in vitro* [5]. Since microtubules are part of the cytoskeleton in almost every eukaryotic cell, the effect of colchicine at the cellular level is generally assumed to stem from its direct interaction with microtubules. Specifically, its anti-inflammatory effect has been attributed to its disruption of microtubules in neutrophils, thereby inhibiting their migration toward the chemotactic factors. Furthermore, Cronstein *et al*. [6] showed that colchicine may also alter the distribution of adhesion molecules on the surface of both neutrophils and endothelial cells, leading to a significant inhibition of interaction between white blood cells (WBC) and endothelial cells interfering with their transmigration. Thus, there is growing evidence that the anti-inflammatory effect of colchicine is multifaceted.

In the present study we tried to better understand the molecular basis of the action of colchicine by analysing the effect of this drug on global gene expression. We exposed HUVEC (human umbilical vein endothelial cell line) cells to different concentrations of colchicine and examined their transcriptional response using complementary deoxyribonucleic acid (cDNA) microarrays. This approach enabled us to take a global view of the transcriptional response induced by colchicine treatment and allowed for the identification of sets of genes that are potentially related to its mechanism of action, beyond its direct interaction with microtubules.

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Materials and methods

The HUVEC cell line

The HUVEC cell line was purchased from Cambrex Bio Science (Walkersville, MD, USA). Cells were grown on EGM-2 medium and their doubling time ranged between 12 and 48 h. The cells were taken at least 5 days after their last split before exposure to colchicine.

Colchicine

Colchicine was purchased from Sigma-Aldrich (St Louis, MO, USA) and was dissolved in the same medium as used for maintaining the cell line.

Study design

The HUVEC cells were grown on culture dishes (10 cm) Falcon (BD Biosciences, Bedford, MA, USA). They were exposed to three concentrations of colchicine: 10 ng/ml (0.025 mM, approximately the plasma therapeutic level), 100 ng/ml (0.25 mM) and 1 mg/ml (2.5 mM) at the following time points: 0, 30 and 120 min, 12 and 24 h. Following the appropriate time of exposure, the cells were immediately harvested using TriZOL (Invitrogen Life Technologies, Carlsbad, CA, USA) and kept at -80°C until ribonucleic acid (RNA) was extracted from all the samples.

Controls of HUVEC cells, using the same conditions and time points but without exposure to colchicine, were obtained and processed similarly. Three replicates were included for the zero time point with no colchicine and duplicates for most colchicine concentrations and time points.

Arrays

DNA microarrays are based on IMAGE clones prepared by the Research Genetics Corporation (Huntsville, AL, USA) [7]. The microarrays we used comprised 42,749 elements (42K) representing 32,275 unique Unigene clusters (build no 158, released 18 January 2003) and 11,946 known genes (unique Unigene symbols). All arrays were printed at the Stanford University School of Medicine according to the Brown lab's protocols in the Stanford Functional Genetics Facility (SFGF).

Isolation of RNA

Total RNA was isolated from the HUVEC cell line using the TriZOL (Invitrogen Life Technologies) reagent according to the manufacturer's protocol. The extracted RNA was further purified using the RNeasy kit purchased from Qiagen (Valencia, CA, USA) and amplified using the Ambion amplification kit (Austin, TX, USA) according to the manufacturer's protocol. The RNA quality was assessed by examining the size distribution of the 28S and 18S ribosomal RNA bands on 1% agarose gel electrophoresis.

Labelling and hybridization

Three to four milligrams of amplified RNA were used for labelling with Cy5-dUTP and subsequent hybridization. Amplified RNA was reverse transcribed with Superscript II (Invitrogen Life Technologies) using random primers (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Each sample was compared with a common reference labelled with Cy3-dUTP (Strategene, La Jolla, CA, USA) as described previously [8–11]. Fluorescent dyes were purchased from Amersham Pharmacia Biotech. Hybridizations were carried out using the standard protocol described previously [10, 12]. Experimental details can be found at http://brownlab.stanford.edu/protocols.html.

Data analysis and clustering

Data were analysed using GenePix 5.0 software (Axon Instruments Foster City, CA, USA). Spots with aberrant measurements due to array artefacts or poor quality were manually or automatically flagged and removed from further analysis. A filter was applied to omit measurements where fluorescent signal from the DNA spot was less than 50% above the measured background fluorescence surrounding the printed DNA spot in both the Cy3 and the Cy5 channels. Genes that did not meet these criteria for at least 80% of the measurements across the samples were excluded from further analysis. Our data were stored in the Stanford Microarrays Database (SMD) [13]. For further analysis we retrieved the $\log_2$ ratios of the background-corrected intensities, i.e. $\log_2$(Cy5/Cy3). We only included genes whose expression varied significantly across the dataset. Specifically, in at least three samples the expression levels of a gene had to differ more than 3-fold from the mean expression level of all samples. Hierarchical clustering was applied to the genes and arrays, using the Pearson correlation coefficient as a measure of similarity and average linkage clustering as described previously [10, 12, 14, 15]. Results were visualized using Eisen's TreeView [14] and custom-made software written in Matlab (http://www.mathworks.com/). The entire dataset and the results of our analysis are available at our web site at http://genome-www.Stanford.edu/.

Significance analysis of microarrays (SAM)

SAM is a statistical approach to the identification of genes whose expression patterns are significantly associated with specific characteristics of the samples [16]. We applied SAM to our dataset in order to identify differential expression patterns between different types of samples. We compared all the samples treated with colchicine versus all untreated samples, treated samples of the early time points (0, 30, 120 min) against non-treated samples of the same time points, and treated samples versus the untreated samples taken at late time points (12 and 24 h).

Gene ontology term analysis

We identified gene ontology (GO) terms enriched with genes whose expression changed significantly under exposure to colchicine. To this end we defined three mean expression profiles over samples without treatment (WT), with short treatment (ST) (30 or 120 min) and with long treatment (LT) (12 or 24 h), each average comprising four samples. We identified the sets of genes whose expression changed by more than a factor of 1.5 between any of these mean profiles. We compared the resulting six gene sets (containing up- and down-regulated genes, respectively, for WT *vs* ST, WT *vs* LT and ST *vs* LT) with all human GO terms for biological process, molecular function and cellular component (downloaded from http://www.geneontology.org in January 2005). Gene enrichment was quantified using P values according to the hypergeometric distribution.

Comparison with patterns of response to other stimuli

In order to gain a better insight into the pattern of response of the HUVEC cells exposed to colchicine, we searched the SMD to find similar studies examining the response of cell lines to different stimuli. We analysed changes in gene expression in HUVEC cells deprived of serum and coronary artery endothelial cells under

conditions of hypoxia (2% oxygen or no oxygen) and compared them with our colchicine dataset.

Results

Exposure of the HUVEC cell line to colchicine at a concentration of 10 ng/ml did not have any significant effect on gene expression in these cells (data not shown). Higher doses of colchicine (100 ng/ml and 1 μg/ml) resulted in significant and reproducible changes. We first wanted to identify which exposure times of colchicine and which concentrations resulted in similar changes in expression. To this end we clustered 12 different samples according to their similarity in expression over all genes in the dataset (Fig. 1). We found three major clusters: (i) samples with LT colchicine treatment (12 or 24 h), (ii) samples with ST colchicine exposure (30 or 120 min), and (3) samples with no treatment at all (WT). Although samples treated with the same concentration and exposure time (i.e. repeats) were the most similar, the differences between the samples for 100 ng/ml and 1 μg/ml colchicine were rather small.

In order to obtain a first impression about the genes affected by short and long colchicine treatment, we performed a GO term enrichment analysis (see Materials and methods for details). This analysis revealed that many of the genes whose expression was altered after ST are involved in the cell cycle and its regulation. More interesting, however, with respect to the anti-inflammatory action of colchicine was the observation that according to the GO annotations many genes that were up- or down-regulated after LT were localized to the cytoskeleton (microtubules, actin filaments, etc.). Therefore, we decided to concentrate our analysis on the expression changes induced by long treatment with colchicine. First, in order to identify the genes whose expression was changed due to colchicine treatment rather than time- and/or medium-dependent factors, we averaged the triplicate samples of zero time with no colchicine exposure and subtracted this value from all the samples. Then, we subtracted the values of the non-treated samples of 12–24 h from the colchicine-exposed samples of the same time points ('mock reduction'). A cluster analysis of these samples revealed two main clusters, one of (about 90) suppressed genes and the other of (about 100) over-expressed genes (Fig. 2). Further inspection of these clusters disclosed that many of the genes were involved in neutrophil migration or other inflammatory processes (Fig. 2b and c). These genes could be roughly grouped into three different categories: (i) genes whose products are related to the cytoskeleton, (ii) genes coding for transcription or growth factors and (iii) genes associated with inflammation (Table 1). Unavoidably, this subgrouping is somewhat artificial since several genes have more than a single function and therefore should be associated with more than one group. Moreover, it should be noted that some genes could not be categorized either because of lack of annotation about their cellular function or because they were not directly related to inflammation or neutrophil migration at the transcriptional level. Among the suppressed genes were those coding for caspase-1, eNOS3, tubulin-beta-5, PI3K, CDC42, BPA and MAPK 1, all of which are likely to participate in inflammation and neutrophil migration as well as in many other intracellular functions (Table 1). Similarly, many of the induced genes (like those encoding moesin, nexilin, podocalyxin and EML-1) are related to the cytoskeleton gene category. Another interesting finding is the significant suppression of many interferon-induced genes which may also be involved in inflammatory processes (Table 2).

We also performed a SAM to reveal genes that changed significantly following 12–24 h of colchicine exposure [16]. SAM identified 300 genes (with a false discovery rate of less than 3%). Among them were almost all the genes associated with inflammation which emerged from the hierarchical clustering (data not shown). Importantly, we did not observe a significant change in the expression of these genes following 30 and 120 min of colchicine exposure.

Comparison of the gene expression profiles of HUVEC cells exposed to colchicine with that of HUVEC cells following serum deprivation or the hypoxic coronary artery endothelial cell line (2% and no oxygen) disclosed that there was little correlation between the transcriptional responses to the three different stimuli (unpublished data).

Discussion

Colchicine is an alkaloid which is used in several inflammatory diseases especially in gout, FMF and Behçet's disease. Colchicine was the first drug known to bind tubulin [17]. Evidence has been presented for the interaction of colchicine with both α and β tubulin monomers [18]. Following this interaction, colchicine induces a conformational change which prevents curved tubulin from adopting the straight structure needed for the assembly with other tubulin monomers in order to form the whole microtubule. Thus, colchicine may inhibit tubulin assembly thereby destabilizing the microtubules [19].

In many inflammatory diseases, neutrophils are the pivotal cells involved. Their participation in inflammation depends upon their ability to migrate towards the damaged or stimulated tissue [20, 21]. Since neutrophil migration is affected by microtubules, the interaction between colchicine and tubulin distorts this capability, thereby suppressing the inflammatory process.

In the present study we wished to better understand the mechanisms by which colchicine exerts its blockage of neutrophil migration. We tried to find out whether an additional anti-inflammatory effect of this drug might be induced at the level of transcription or signal transduction rather than by the direct interaction between colchicine and tubulins.

Since endothelial cells play an important role in neutrophil transmigration towards the inflammatory site, and since it was shown that colchicine can alter the distribution of adhesion molecules on these cells [6], we chose to test the genome-wide changes in gene expression in HUVEC cells (endothelial cell line) following exposure to colchicine.

An unsupervised cluster analysis revealed very distinct transcriptional responses after short and long exposure times (Fig. 1). Since genes known to be involved in inflammation were altered mainly after long colchicine exposure, we analysed these genes in more detail, focusing on significantly induced and suppressed genes (Fig. 2). Among the approximately 90 most strongly suppressed genes, 32 could be grouped into three main families: genes related to inflammatory processes, genes associated with the cytoskeleton and genes encoding transcription and growth factors (Table 1). Among the top 100 over-expressed genes, 10 were found to be related to inflammation and were grouped in the cytoskeleton and transcription families. In many cases genes could not be related directly to inflammation or neutrophil migration, and in others reliable functional annotation is still missing.

We believe that the inference of a change in expression of these genes upon colchicine treatment is reliable for the following reasons. First, for most genes, we could see a gradual change of expression over a few time points and, significantly, a stronger change in expression for a higher concentration of colchicine. Second, checking microarray experiments performed as duplicates or triplicates in general revealed consistent results under the same conditions. Third, three complementary methods of analysis (unsupervised hierarchical clustering, GO annotation and SAM) yielded similar results.

A complex network of interrelated signalling systems controls the motile response of neutrophils and mediates their morphological and functional polarity [20, 21]. These complex events are conducted by many enzymes and compounds, among which protein kinase 3 (PI3K) and the cytosolic guanosine triphosphate (GTP)-binding proteins RHO and Rac are key regulators. PI3K

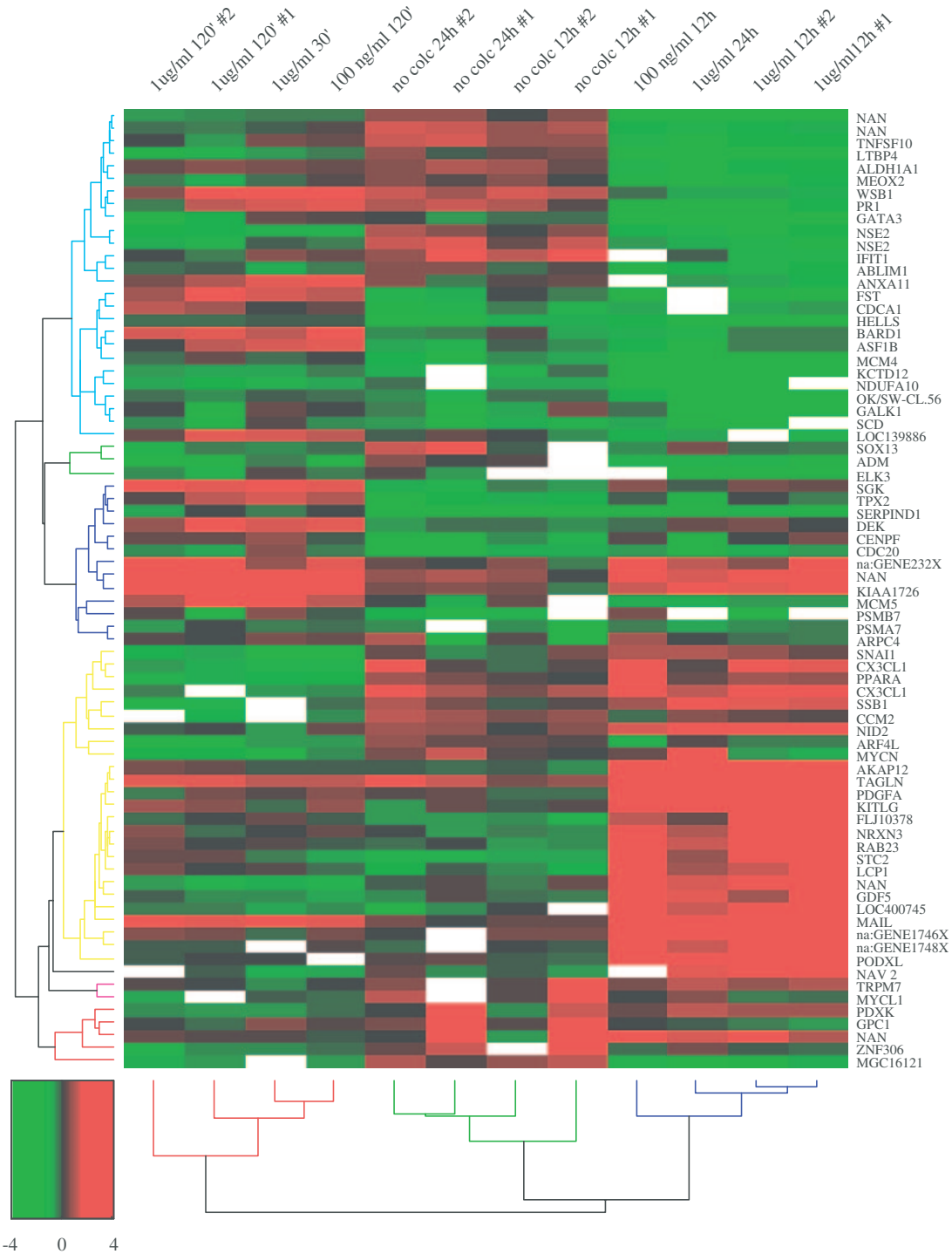

FIG. 1. Clustered expression profiles. Each column corresponds to one of 12 samples with different exposure times and concentrations of colchicine (as indicated). The samples were clustered using average linkage and Pearson correlations over all genes in the dataset. Expression levels for a variable selection of 75 genes (rows) are shown and colour coded according to the colour bar. Note the emergence of three main clusters containing samples with no, short and long colchicine treatment.

activation results in increased production of phosphatidylinositol 3,4,5-triphosphate. These lipid products regulate localization and possibly stabilization of actin filaments—two activities which are prerequisites for neutrophil migration. They define neutrophil polarity and stabilize the generation of pseudopodia at their opposite pole [22, 23]. GTP proteins of the RHO family and mitogen-activated protein kinase (MAPK) are also activated and involved in this process [24]. Another protein which is activated in the process of Polymorphonuclear cells (PMN) migration is CDC42 [25]. CDC42 was shown to be important in stabilization of polarity and consolidation of the leading edge and efficient PMN migration. Cells lacking this protein form unstable pseudopods and migrate poorly. While screening the gene cluster suppressed by colchicine after 12–24 h exposure, we realized that many of its genes encode the above compounds (PI3K, CDC42, MAPK1). Furthermore, other proteins from the RHO family as well as genes related to tubulin (tubulin-5-beta) and actin filaments were also suppressed (Table 1). This suggests that inhibition of

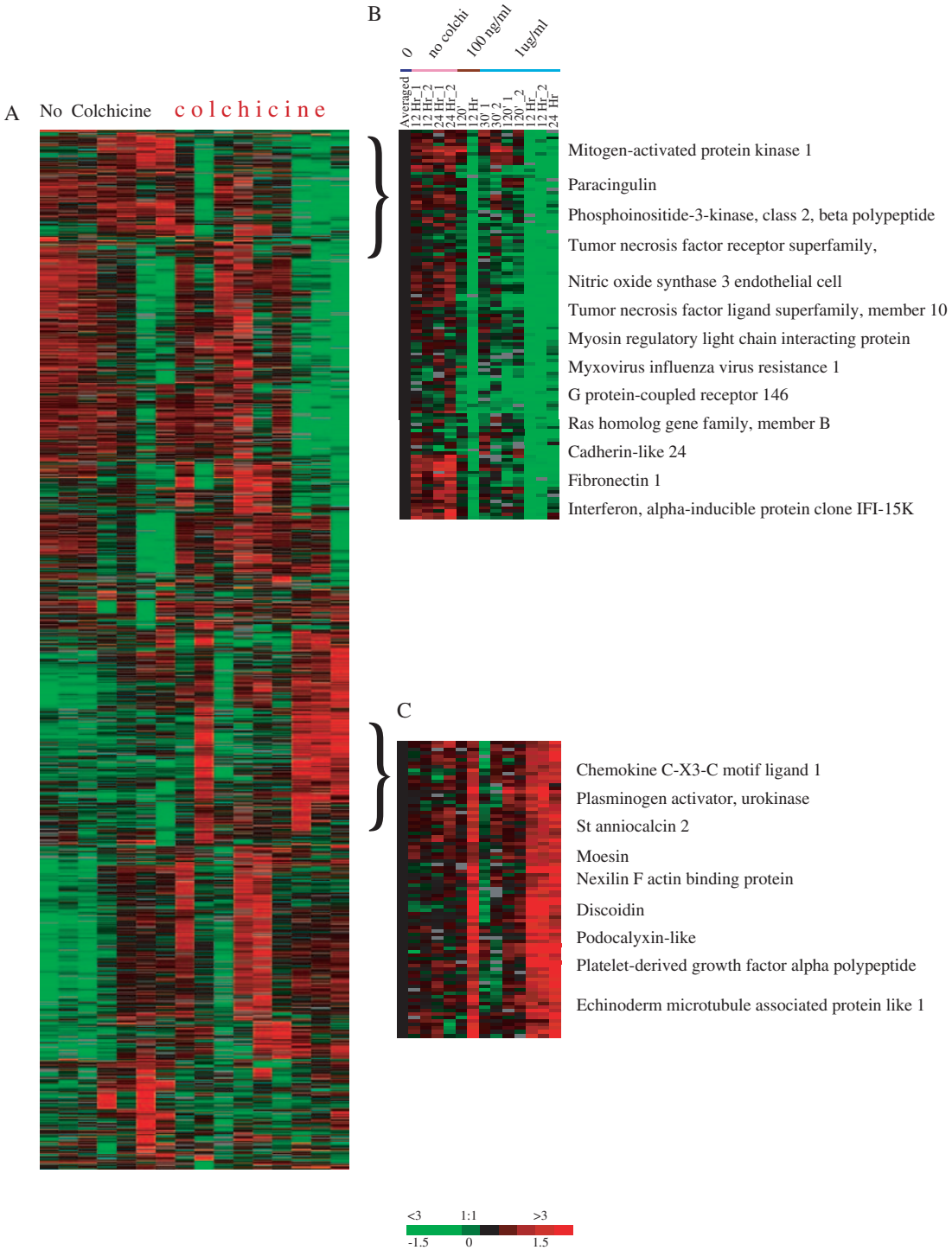

FIG. 2. Time-course of gene expression changes in HUVEC cells following exposure to colchicine. Panel A: The left-most seven columns represent HUVEC cell samples which were not exposed to colchicine whereas the nine right-most columns are of cell samples with the drug. Two distinct clusters of genes induced (red) or suppressed (green) by colchicine are shown. It is clear that the significant changed occurred after 12 or 24 h whereas no significant change was observed following a shorter time of exposure. Moreover, changes were evident only following exposure to colchicine concentrations higher than 100 ng/ml. (The left-most three columns represent zero time samples with no colchicine exposure). Panel B shows some of the genes suppressed following exposure to colchicine. Panel C shows some genes which were over-expressed following exposure to colchicine. In panels B and C, the left black column represents zero-averaged triplicate of zero-time no-colchicine samples.

chemotaxis by colchicine may not be just a pure mechanistic blockage of microtubules but may be affected indirectly by suppression of many genes encoding essential proteins required for PMN migration.

Several genes which may have a role in other aspects of inflammation were also suppressed by colchicine. Caspase-1 is a cysteine aspartic acid protease which cleaves pro-IL-1β releasing the mature cytokine which is involved in a variety of inflammatory

Table 1. Groups of genes suppressed or induced following exposure to colchicine

Gene group	Gene name or symbol
Inflammation	*CASP1*
	GPR146
	NOS3
	G1P2
	IFIT1
	MX1
	IFITM1
	GBP1
	TNFSF10
	CTSC (cathepsin C)
	CTSZ (cathepsin Z)
	Plasminogen activator, urokinase
Cytoskeleton (tubulin, actin, adhesion molecules)	Tubulin 5 beta
	Tubulin beta 2
	CDC42BPA
	MYLIP
	Fibronectin
	Paracingulin
	PIK3C2B
	MAPK1
	RHOB
	RHOC
	RHOJ
	NSE2
	Cadherin-like 24
	Discoidin
	Nexilin
	Echinoderm
	Podocalyxin
	Moesin
	Coronin
	DUSP8
	Stanniocalcin 2
Transcription and growth factors	*TGFBR3*
	GATA3
	TIMP1
	PPP1CA
	Hoxa 5
	Actin-binding LIM
	STAT1
	APBB2
	AKAP12

The induced genes are in bold type.

Table 2. Effect of colchicine on interferon-associated genes

Gene	Effect
IFIT1	↓
IFITM1	=
IFIH1	=
IFI30	↓
IRF6	=
ISG20	=
G1P2	↓
Myxovirus 1	↓
GBP1	=↓

=, no change; =↓ or ↑, mild change.

processes [26]. Since colchicine suppressed the gene encoding this protein, release of mature IL-1 is reduced, thereby inhibiting the propagation of inflammation. Nitric oxide synthetase 3 (eNOS3) is an endothelial enzyme producing nitric oxide which is implicated in relaxation of vascular smooth muscle [27]. Its lack may lead to relative vasoconstriction, which may reduce the blood supply to the inflammatory site. It can also affect the transmissibility of the endothelial walls to neutrophil transmigration. Thus suppression of the eNOS3 gene by colchicine may further contribute to restraint of inflammation.

G1P2, *MX1* and *IFIT1* are genes encoding interferon-induced proteins. G1P2 showed a specific chemotactic activity towards neutrophils and may activate them to induce release of other chemotactic factors [28]. MX1 (mixovirus resistance 1 interferon inducible protein) is a member of the GTPase dynamin family. These proteins are involved in many processes including remodelling cellular membranes and cytokinesis through their interaction with actin filaments [29]. Furthermore, since interferon may induce production of tumour necrosis factor-α (TNF-α) production, its down-regulation may also contribute to the suppression of inflammation [30]. Thus, the present study discloses a general suppression of many compounds induced by interferon (Table 2), suggesting additional pathways through which colchicine may restrain the inflammatory process.

As already mentioned, some of the genes associated with the cytoskeleton were induced by colchicine exposure. Podocalyxin-like protein (PODXL), a sialomucin-type member protein, is one of them. This protein was first described in glomerular podocytes and endothelial cells [31]. It can function as an antiadhesin that maintains open filtrating foot processes in the podocytes by charge repulsion. However, it has also been described as having an adhesion function resembling that of L-selectin. Since this molecule has two opposing effects, it is not clear what the exact outcome is following colchicine exposure. However, it is still possible that the net effect may be anti-inflammatory.

Another gene induced by colchicine encodes EML1 (echinoderm microtubule associated protein like-1). This protein may modify the assembly dynamics of microtubules by shortening them due to lateral destabilization and thus affecting cytokinesis and cell migration [32]. Therefore, the induction of this gene by colchicine may also suppress inflammation.

Several questions maybe raised following the above observations. Do the changes in gene expression reflect a general response to a 'stressful stimulus' such as cell-induced toxicity by colchicine? Do these changes result from a direct action of colchicine on transcription or through its effect on tubulins and microtubules?

The doses of colchicine used in the current study, although higher than the plasma therapeutic dose, were not high enough to cause cell intoxication. First, Chappey *et al.* [33] measured the levels of colchicine concentrations in white blood cells. They found that the level of the drug was 60–600 times higher in the neutrophils compared with the plasma. In the present study, we exposed the cells to 100 ng/ml and 1 mg/ml (final concentration), about 10 to100 times the plasma therapeutic dose, respectively. Second, if these changes reflected toxicity, we should have seen (in contrast to our observation) dying cells since they become detached from the culture dish and can be easily recognized. Third, if the assumption is that colchicine induces a general 'stress response', such as in the case of exposure to hypoxia or serum deprivation, we would expect an identical pattern of cell response. However, we found that each of these stimuli induced distinct patterns of gene expression.

There is no evidence to support the view that colchicine can alter gene expression directly, although it cannot be excluded. However, the fact that colchicine may interact with microtubules alludes to the possibility that it has an indirect effect on transcription through the disruption of the cytoskeletal infrastructure. In fact, our initial GO analysis revealed that colchicine affected many groups of genes with a common annotation following both short and long time exposure. This observation supports the view that colchicine exerts its effect indirectly, through disruption of the cytoskeletal infrastructure for transcription rather than by direct regulation of each affected gene.

Based upon current knowledge and the present results, we propose the following model for the anti-inflammatory action of colchicine (Fig. 3). We hypothesize that there are two mechanisms of action. The first mechanism is through its direct interaction

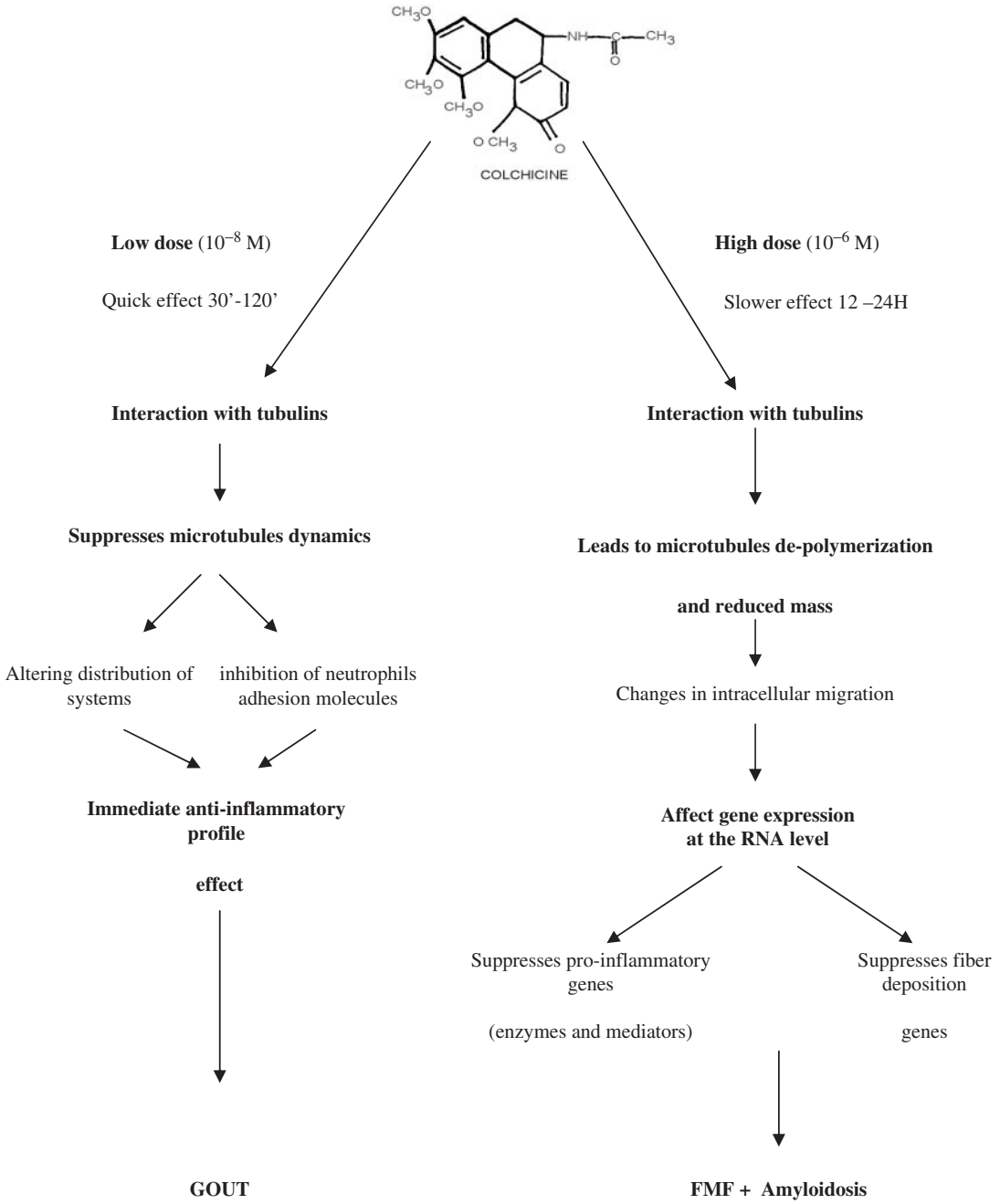

FIG. 3. A model of the mechanism of action of colchicine. The left arm shows the relatively quick effect of low concentration of colchicine through suppression of microtubules dynamics. The right arm depicts the effect of higher concentrations of colchicine through microtubules depolymerization and mass reduction. The latter activity requires 12–24 h.

with tubulin monomers or with whole microtubules leading to their conformational changes and destabilization. This is a purely mechanistic (physical) interaction which may affect neutrophil migration and alter distribution of adhesion molecules on neutrophils and endothelial cells. The concentration needed for these effects is relatively low (plasma levels about 10^{-8} M) and the time required is between about 30 and 120 min. The second mechanism of anti-inflammatory activity of colchicine requires a higher concentration of the drug (about 10^{-6} M) and a longer time (about 12–24 h). It is mediated by the depolymerization of microtubules with reduction in their mass and length. This effect may alter the levels of expression of many genes through a transcriptional response. Thus, suppression of enzymes such as caspase-1, eNOS3 or other mediators of chemotaxis may lead to inflammatory restraint. The above hypothesis is in accord with the findings of Panda *et al*. [34], who showed that different concentrations of colchicine exert diverse activities on the stabilization, dynamics and mass of microtubules.

The model we propose here can explain the clinical observation that colchicine is ineffective in relieving ongoing acute FMF attacks while being very efficient in preventing the attacks when given chronically. It seems that colchicine exerts its prophylactic activity through gene suppression which requires 12–24 h and a higher dose of the drug as maintained in neutrophils and probably other cells. In cases of acute gout (local synovitis), the immediate response to colchicine therapy relies on its quick effect on neutrophil motility and in altering the distribution of adhesion molecules on polymorphonuclear and endothelial cells surfaces [6].

This effect is achieved by lower colchicine concentration and requires a shorter time.

Colchicine is also known as an effective agent in preventing the development of amyloidosis in FMF patients, and even in regressing it when the amyloid fibres have already been deposited [35]. The traditional explanation for this effect is that since amyloidosis is secondary to the chronic inflammatory conditions the restraint of this process by colchicine prevents the deposition of the serum amyloid A (SAA) fibres. The present study may offer an alternative explanation, whereby a direct suppression of genes, such as fibronectin, by colchicine, averts the assembly and deposition of fibres such as amyloid. This possibility may have a wide implication, since it can explain the beneficial effect of colchicine in other conditions of fibre deposition such as in scleroderma and cirrhosis where no direct relationship with the intensity or chronicity of an underlying inflammation exists.

Finally, the fact that colchicine suppresses interferon-induced genes—recently shown to be over-expressed in systemic lupus erythematosus (SLE)—raises the possibility that it may have a beneficial effect in this disease as well [36, 37].

A number of limitations of the study should be noted. First, we did not test a control medication such as one of the NSAIDs and its effect on gene expression in HUVEC cells. Theoretically this could add to our findings. However, trying to elucidate the mechanism by which colchicine exerts its anti-inflammatory effect, we checked whether it has an anticyclo-oxygenase (Cox I or II) inhibitory effect. We found that it does not have any suppressive effect on these enzymes [38]. This may suggest that colchicine and NSAIDs may have different effects on gene expression profiles. Second, for technical reasons, we tested in the present study the effect of colchicine on HUVEC cells which represent endothelial cells. While ideally one would like to study the effect of colchicine on neutrophil gene expression, our current results are nevertheless highly informative since both kinds of cells have a similar repertoire of expressed genes. Finally, we have to remember that the microarray tool should be treated as a large-scale screening tool. In order to make definitive conclusions concerning the exact intracellular effect of colchicine it is necessary to perform *in vitro* studies, especially at the protein level.

Rheumatology	Key messages
	• Colchicine treatment affects global gene expression in HUVEC cells. • The genes induced or suppressed by this drug may play a major role in inflammatory processes, neutrophil migration and mechanisms of fibre deposition. • These effects of colchicine require a higher concentration of the medication and a longer time of exposure.

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The authors declare no conflicts of interest.

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