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Increased chloride efflux in colchicine-resistant airway epithelial cell lines

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Abstract

Colchicine has been proposed as a treatment to alleviate chronic lung inflammation in cystic fibrosis patients and clinical trials are ongoing. Our aim was to investigate whether chronic exposure of cystic fibrosis cells to colchicine can affect their ability to transport chloride in response to cAMP. Colchicine-resistant cells were selected by growing in medium containing nanomolar concentrations of the drug. While microtubuli were affected by acute exposure to colchicine, they appeared normal in colchicine-resistant cells. Colchicine-resistant clones had higher expression of multidrug resistance proteins compared to untreated cells. Cystic fibrosis transmembrane conductance regulator (CFTR) labelling by immunocytochemistry showed no significant changes. The intracellular chloride concentration and basal chloride efflux of the cystic fibrosis treated cells increased significantly compared with untreated cells, while for the cAMP-stimulated Cl-efflux there was no significant change. The results suggest that colchicine promotes chloride efflux via alternative chloride channels. Since this is an accepted strategy for pharmacological treatment of cystic fibrosis, the results strengthen the notion that colchicine would be beneficial to these patients.
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Keywords: Cystic fibrosis; Colchicine; Multidrug resistance; Airway cells; MQAE; Chloride efflux

1. Introduction

Cystic fibrosis (CF) is the most common autosomal recessive lethal inherited disease, affecting 1 in 3000 Caucasians, and is characterised clinically by chronic obstructive lung disease, pancreatic insufficiency, male infertility and high levels of salt in the sweat, which is a diagnostic feature. The disease is caused by mutations in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride and bicarbonate channel regulated by cAMP, localised in the apical membrane of epithelial cells. In addition, CFTR regulates other ion channels. So far, over 1000 mutations of CFTR have been described, and a single mutation (ΔF508) accounts for more than 70% of all cases [1].

In the airways, the defective or absent CFTR leads to a defective chloride secretion of the epithelial cells in response to cAMP elevating signals, as well as to an increased absorption of sodium through the respiratory epithelia. This results in dehydrated mucus, defective ciliary clearance, pathognomonic colonisation with pathogens such as *Pseudomonas aeruginosa*, chronic inflammation and infection.

Colchicine has long been known for its anti-inflammatory effects in several diseases such as acute gouty arthritis, familial Mediterranean fever, and other rheumatic and non-rheumatic conditions [2]. The interest for colchicine in cystic fibrosis is double-sided. On one hand, its anti-inflammatory and anti-fibrotic effects may be useful in the treatment of the chronic inflammation characteristic for the lung disease. Indeed, a clinical trial of chronic administration of colchicine in a small number of patients has shown improvements in the lung condition [3,4]. On the other hand, colchicine, as other chemotherapeutic drugs, induces a multidrug resistance phenotype characterised by the up-regulation of several proteins such as multidrug resistance protein (MDR) and multidrug-related protein (MRP). These proteins are active pumps, belong to the

Abbreviations: Calu-3, normal submucosal epithelial cells; CF, cystic fibrosis; CFBE, cystic fibrosis bronchial epithelial cells; CFSME, cystic fibrosis submucosal epithelial cells; CFTR, cystic fibrosis transmembrane conductance regulator; IBMX, 3-isobutyl-1-methylxanthine; MDR, multidrug resistance proteins; MRP, multidrug-related proteins

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same family of protein as CFTR, the ATP-Binding Cassette (ABC) proteins and are involved indirectly in the movements of ions across membranes [5].

The physiological function of the MDR1 protein may include modulation of pH-dependent Cl^- transport (possibly also volume and intracellular pH regulation) in proximal tubule cells of the kidney [6]. MDR1 is inhibited by glibenclamide and related compounds, as is CFTR [7]. A CF patient treated with chemotherapeutic drugs for a fibrosarcoma was reported to show potent and persistent improvement of the lung function [8], and it was suggested that this was correlated with the multidrug resistance phenotype compensating for the non-functional CFTR.

There is an abundance of studies on the effect of the MDR protein overexpression and multidrug resistance phenotype on ion transport (directly or indirectly) in various cells [9–13], but very little is known about the effect of colchicine on the airway cells relevant for cystic fibrosis. In this study, we addressed the question whether colchicine in the nanomolar range concentration can induce a multidrug resistance phenotype and whether it can improve the chloride transport in three airway epithelial cell lines.

2. Materials and methods

2.1. Cells

Cystic fibrosis human bronchial epithelial cells CFBE41o- (CFBE, homozygous for the ΔF508 mutation) and the cystic fibrosis submucosal epithelial cell line CFSMEo- (CFSME, genotype ΔF508/unknown), both kind gifts of Dr. D. Gruenert (San Francisco, USA) were cultured in adherent flasks (Sarstedt) in Eagle's minimal essential medium with Glutamax (SVA) supplemented with 10% foetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin sulphate. The normal human serous cell line Calu-3 (ATCC) was grown in a similar medium, containing 1 mM sodium pyruvate and 1% non-essential amino acids (both from Sigma). The human colonic adenocarcinoma cell line T84 was grown in DMEM:Ham's F-12 (SVA) supplemented with 6% foetal calf serum, 15 mM HEPES, 100 IU/ml penicillin and 100 μg/ml streptomycin sulphate.

All cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% air, and the medium was changed twice weekly. Colchicine-resistant cells were obtained by culturing the original cells until confluence in medium containing 0.5 nM colchicine (Sigma), the maximal concentration without significant cytotoxic effects. The drug concentration was incrementally increased by 0.5 nM after each confluent passage, up to 6 nM colchicine in the case of Calu-3 and 4 nM for CFBE and CFSME cells. Colchicine was present in the medium at all times except during the chloride efflux experiments (which lasted on average 30 min). Thus, the colchicine-resistant cells were a population of selected cells. During the course of the experiments, several series of colchicine-resistant cells were obtained, starting from different passages of the untreated cells. Their appearance and responses were homogenous, with no differences among series. As controls we used cells from the same passage number as those resistant to colchicine.

2.2. Cytoskeleton

For immunostaining of the cytoskeleton, untreated and colchicine-resistant cells were grown on glass coverslips until confluent and rinsed with 37 °C warm phosphate buffer saline (PBS, from SVA). In order to study the effect of acute exposure to colchicine, untreated cells were grown on glass coverslips until 70% confluent and then treated with 3 nM colchicine for 48 h. Cells were fixed in 37 °C warm cytoskeleton stabilising buffer (10 mM HEPES pH 6.9, 138 mM KCl, 3 mM $MgCl_2$, 2 mM EGTA, 0.2% Triton X-100) with 4% paraformaldehyde for 20 min. Then, unspecific epitopes were blocked with 1% bovine serum albumin (BSA) in TBS (150 mM NaCl, 10 mM Tris–HCl pH 8.0) containing 0.05% Tween-20 (TBST-BSA) for 30 min. The glass slides were incubated for 1 h with the monoclonal mouse antibody anti-α-tubulin (Sigma) diluted 1:200 in TBST-BSA.

After rinsing, the cells were incubated for 1 h in the goat anti-mouse rhodamine-conjugated secondary antibody (Southern Biotechnology Associates Inc.) diluted 1:50 in TBST-BSA and 5 μg/ml Phalloidin-FITC (Sigma). The coverslips were rinsed and mounted in 90% glycerol, 2% *N*-propyl-gallate, pH 9 containing 1 μg/ml 4′,6-diamidino-2-phenylindole-dihydrochloride hydrate (DAPI), and sealed with transparent nail polish.

Pictures were taken with a fluorescence microscope equipped with a digital camera (Leica Microsystems Ltd.) using identical settings and appropriate filters for the collection of fluorescence. Illumination was obtained from a xenon lamp; the green fluorescence of actin was produced with excitation light of 480 ± 15 nm, and emitted light collected at 535 ± 20 nm. The red fluorescence of tubulin was produced with excitation light of 535 ± 20 nm and the emitted light collected through a 590 nm LP filter. The exposure and compensation parameters of the digital camera were saved and used at different times such that pictures were taken under identical conditions.

2.3. CFTR immunodetection

For immunostaining of CFTR, cells were grown on glass coverslips until confluent and rinsed with cold PBS. The cells were fixed in methanol at −20 °C for 5 min, rinsed with TBS, permeabilised with 0.2% saponin for 5 min, then incubated with the mouse monoclonal MATG-1061 anti-CFTR antibody (Transgene) diluted 1:500 in TBS-BSA, for 1 h at room temperature. After rinsing, the cells were incubated with an HRP/Fab polymer conjugate followed by 3-amino-9-ethyl-carbazole (AEC) chromogen

detection (Zymed Laboratories Inc.). The nuclei were counterstained with haematoxylin for 1–2 min and the coverslips were mounted in aqueous medium (Aquatex, from Merck). Pictures were taken with an optic microscope equipped with a digital camera (Leica).

2.4. MDR immunodetection

For immunodetection of the MDR protein, the cells were grown to confluence on adherent plastic flasks and collected by trypsinisation. The cell line T84 was used as positive control. Proteins were extracted with a lysis buffer containing 50 mM Tris–HCl pH 7.4, 10 mM EDTA, 1% SDS and 10 μg/ml proteinase inhibitor cocktail (Sigma) for 30 min on ice, followed by brief sonication. After centrifugation (10 min at 10,000 × *g*), the total protein concentration was determined by a modified Pierce method (Bio-Rad DC Protein Assay, Bio-Rad Laboratories).

Protein samples were diluted in denaturating buffer (100 mM Tris–HCl pH 6.8, 20% glycerol, 15% β-mercaptoethanol, 6% SDS, 0.001% bromophenol blue), incubated for 30 min at 37 °C and 10 μg protein/sample run on a 7.5% SDS–polyacrylamide gel. The proteins were transferred to a nitrocellulose membrane (Schleicher & Schnell GmbH) by electroblotting (1 h at 100 V) and the transfer efficiency was checked by non-specific staining with Ponceau S (Sigma). The blots were blocked (16 h at 4 °C) with TBST containing 2% BSA, and incubated for 2 h with a rabbit anti-hMDR polyclonal antibody (H-241 from Santa Cruz Biotechnology) diluted 1:400 in TBST-BSA.

Specific immunocomplexes were detected using a secondary donkey anti-rabbit biotin-conjugated antibody (Amersham Pharmacia Biotech Ltd.) diluted 1:400 in TBST-BSA followed by incubation with streptavidin–horseradish peroxidase conjugate (Amersham) diluted 1:2000 in TBST-BSA, extensive washing in TBST and autoradiography with the enhanced chemiluminiscent reagent (ECL, Amersham). The molecular weights were estimated with the help of a prestained protein marker (New England Biolabs). Semi-quantitative assessment of MDR expression was done by densitometry of immunoblots radiographs using the ImagePro 4.5 software (Media Cybernetics Inc.).

2.5. Chloride measurements

For the experiments cells were grown to confluence on glass coverslips and loaded with 10 mM MQAE (*N*-(ethoxycarbonylmethyl)-6-methoxyquinolinium bromide, from Molecular Probes) in medium, for 2–4 h at 37 °C. The coverslips were placed at the bottom of a perfusion chamber on the stage of an inverted microscope (Nikon Diaphot). The temperature was kept at 37 °C by heating the chamber holder and the objective separately. The cells were perfused continually with buffer pre-warmed at 37 °C with the help of a peristaltic pump (Ismatec). The exchange volume was 30 mm^3 and the buffer flow 1 ml/min.

The chloride efflux experiments were performed by sequential exposure of the cells to a chloride buffer containing of 140 mM NaCl, 5 mM KCl, 5 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), 1 mM $MgCl_2$, and 5 mM glucose pH 7.4, followed by exposure to a chloride-free buffer of similar composition, but with NO_3^- as the substituting anion. The cells were allowed to recover by perfusing them with the chloride buffer and the cAMP-stimulated chloride efflux was determined by exposure to chloride-free buffer containing 5 μM forskolin and 100 μM IBMX (both from Sigma). The agonists were present in the chloride buffer for 5 min before the efflux.

For the intracellular calibration, a K^+-rich buffer containing 120 mM K^+, 5 mM HEPES, 5 mM glucose, 1 mM Mg^{2+} and various concentrations of Cl^- and NO_3^- was used at pH 7.2, in the presence of nigericin (10 μM) and tributyltin (10 μM) [14]. Double-point in situ calibration was performed. The experiment was ended by recording of the autofluorescence of the cells in a quenching solution containing 150 mM KSCN and 10 mM HEPES, pH 7.2.

A Quanticell 2000 image-processing system (VisiTech International) provided excitation light at 355 nm wavelength (20 nm bandwidth). The emission was measured at 460 nm (30 nm bandwidth). Cells were exposed to excitation light for 16 ms at an interval of 3–10 s and the digitised image was recorded. The fluorescence was displayed as arbitrary units.

The fluorescence was transformed into chloride concentration using the results of the intracellular calibration, as described by Chen et al. [15]. For each experiment, all cells in the optic field were analysed (20–40 cells) and their averaged response was counted as one experimental data point.

Due to the electroneutral characteristics of the chloride efflux in non-excitable cells, the changes in intracellular chloride concentration during the efflux can be approximated by an exponential function. GraphPad Prism 3.0 software (GraphPad Software) was used to determine the parameters of the exponential function fitting the data and the maximal value of the chloride efflux rate. For each colchicine concentration tested, 4–12 measurements were performed and results followed a normal distribution. For the statistics, one-way ANOVA was used, followed by Dunnet's post test for multiple comparison of selected means.

3. Results

3.1. Cytoskeleton

Colchicine is known to have a specific effect on the microtubuli, by inhibiting their assembly at low colchicine concentrations. After progressive exposure of the CFBE cells to increasing concentrations of colchicine the localisation of microtubuli was similar to that in unexposed cells. The microtubuli extended both in control and col-

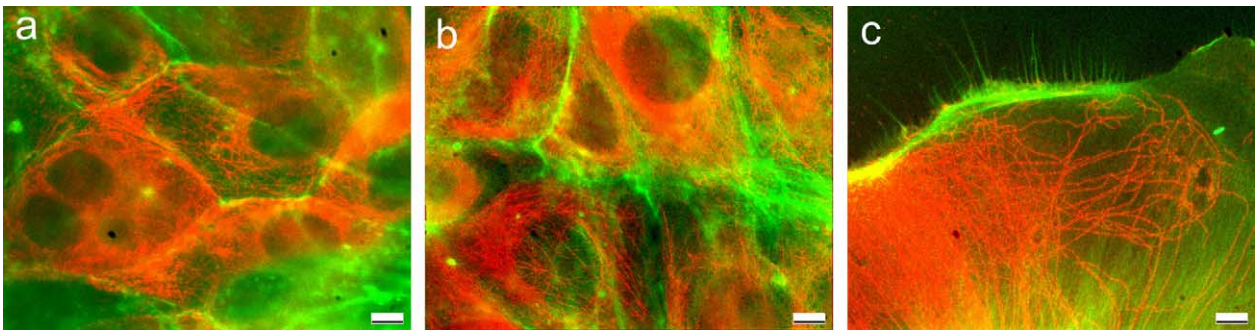

Fig. 1. The effect of colchicine on microtubuli in CFBE cells: (a) control, (b) cells resistant to 4 nM colchicine, (c) cells exposed acutely to 3 nM colchicine for 48 h. The cells were fixed at 37 °C and stained with a monoclonal antibody against tubulin and with FITC-conjugated phalloidin (against actin). Microtubuli staining is shown in red, actin staining is shown in green. Scale bar 10 μm.

chicine-resistant cells from the perinuclear region to the cell membrane (Fig. 1a and b), which is characteristic for an intact microtubuli cytoskeleton. In contrast, acute exposure of the cells to the same nanomolar concentration produced changes in morphology, with enlarged cells (note the scale bar), and shortened and disorganised microtubuli (Fig. 1c). Similar results were observed for the other cell lines in the study (data not shown).

3.2. CFTR localisation

The most intense expression of CFTR was observed as expected in the Calu-3 cells, where the protein was present in the cytoplasm from the perinuclear region to the cell membrane (Fig. 2a and b). The control cystic fibrosis cell lines had a weaker staining for CFTR in the cytoplasm and no visible staining in the cell membrane (Fig. 2c and e). The colchicine-resistant CF cells had a somewhat more intense staining of CFTR in the cytoplasmic compartment, but no staining was observed close to the cell membrane (Fig. 2d and f).

3.3. MDR expression

The MDR protein was identified on the basis of its molecular weight (170 kDa) and its specific immune staining with the anti-MDR polyclonal antibody. The bands had a characteristic smeared pattern and sometimes double bands were visible (Fig. 3a), probably due to alternative forms of the protein caused by differential glycosylation as also noted by other authors [16].

Non-specific protein staining with Ponceau S showed that the MDR is not an abundant protein (data not shown), not even in the colchicine-resistant cells. Semi-quantitative

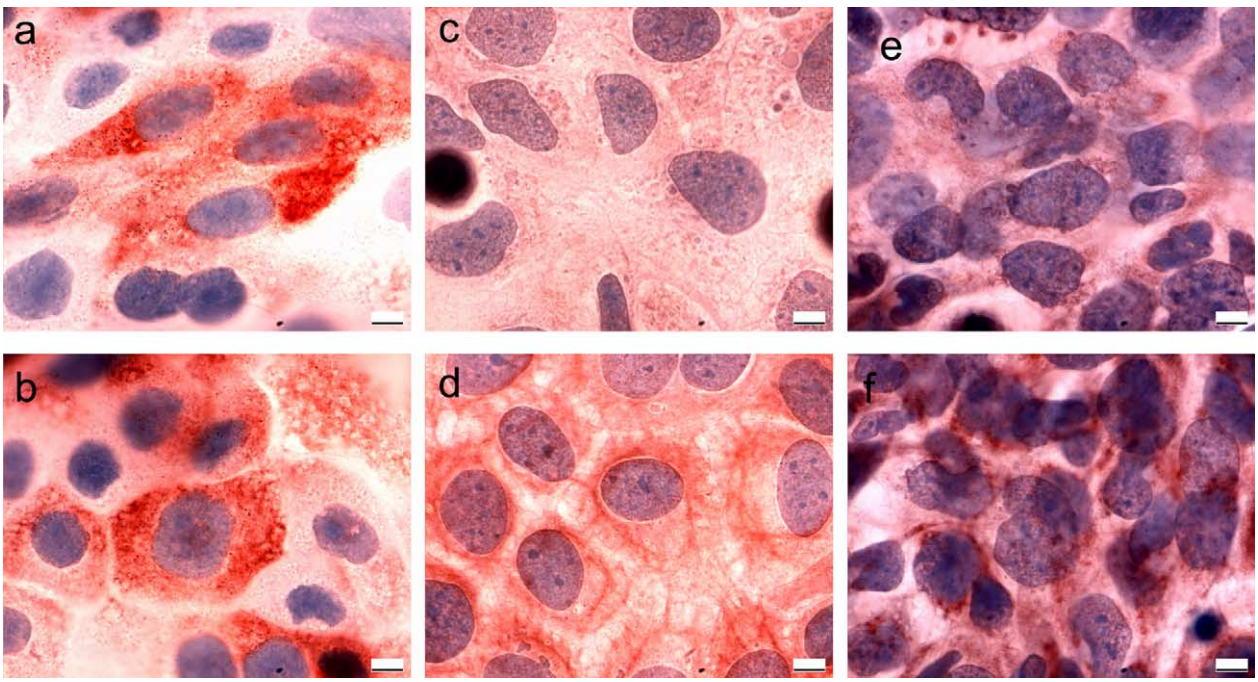

Fig. 2. The effect of colchicine on CFTR immunolocalisation: (a) Calu-3 control, (b) Calu-3 resistant to 4 nM colchicine, (c) CFBE control, (d) CFBE resistant to 2 nM colchicine, (e) CFSME control, (f) CFSME resistant to 2 nM colchicine. The CFTR protein was detected by incubating the fixed cells with a monoclonal anti-CFTR antibody, followed by AEC-chromogen staining (red). The nuclei were counterstained with haematoxylin. Scale bar 10 μm.

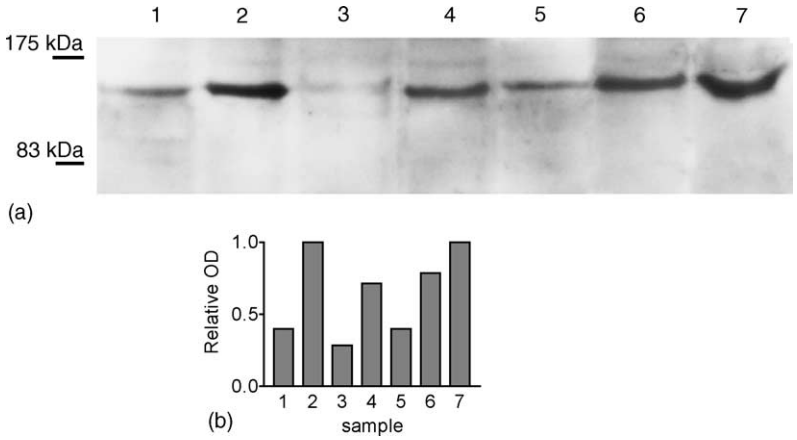

Fig. 3. The effect of colchicine on MDR1 protein expression: (a) representative Western blot, (b) densitometry of the above MDR1 bands showing the relative amount of MDR1 protein. 1: Calu-3 control, 2: Calu-3 resistant to 4 nM colchicine, 3: CFBE control, 4: CFBE resistant to 4 nM colchicine; 5: CFSME control, 6: CFSME resistant to 4 nM colchicine, 7: positive control (T84 cells).

analysis of the MDR expression showed an increase of the protein amount in relation to increased concentrations of colchicine (Fig. 3b). For each cell line, the staining for MDR1 was more intense at higher colchicine concentrations (data not shown).

3.4. Chloride efflux

In all cell lines we noticed with increasing colchicine concentrations, a steady increase in the intracellular chloride concentration in resting conditions and a similar increase in the basal chloride efflux rate, which became significant ($P < 0.05$, ANOVA followed by Dunnet's test) for all cell lines at concentrations higher than 2–4 nM (Fig. 4). The responses of the cells were not homogenous and the results showed a large spread, nonetheless normally distributed.

The stimulated chloride efflux in the presence of the cAMP agonists forskolin and IBMX followed the same pattern, with significant increases in the cells resistant to colchicine at concentrations higher than 2–4 nM compared to control cells ($P < 0.05$). The Calu-3 cell lines had a characteristic response for the wild-type active CFTR, with values of the stimulated chloride efflux much larger than the basal efflux (note the logarithmic scale). For the CF cell lines, there was no difference in the basal and stimulated chloride efflux rate neither for the control cells nor for the colchicine-resistant cells.

High intracellular chloride concentrations tended to be associated with higher basal chloride efflux rates, regardless of the cell line studied (Fig. 5).

4. Discussion

In the present study, we report that airway epithelial cells resistant to colchicine in the nanomolar range had a higher expression of MDR1 proteins compared to the untreated cells, without significant changes in the expression and localisation of CFTR. The basal chloride efflux of the treated cells increased significantly compared with the untreated cells, while for the cAMP-stimulated Cl-efflux there was no significant change. The results suggest that the colchicine treatment may affect the ion permeability of the cell membrane but does not specifically increase cAMP-induced chloride efflux.

Colchicine has long been known and used for the treatment of acute gouty arthritis. Recently the use of colchicine has expanded to other conditions like biliary cirrhosis, familial Mediterranean fever, psoriasis, sarcoidosis, amyloidosis and other rheumatic diseases [2]. Its anti-inflammatory effects are in part explained by a potent inhibition of leukocyte chemotaxis. The usual therapeutic dosage is 1–2 mg/day, with a corresponding plasma concentration of 3 ng/ml (7.5 nM). The concentrations used in this study are also in the low nanomolar range, in good agreement with the clinically tolerated daily dosage.

Of interest for cystic fibrosis is the fact that per os colchicine overdose may lead to a cholera-like syndrome associated with dehydration, shock, and fatal complications [17]. Colchicine at low concentrations inhibits microtubule self-assembly, and at high dosage it can induce complete disassembly of preformed microtubules [18,19]. At the concentration used in our studies, the microtubuli were not affected, as shown by the cytoskeleton immunochemistry, indicating true resistance of the cells to colchicine.

CFTR and MDR proteins are expressed constitutively in the epithelium and glands of the human airway. The expression is more intense in the serous cells of the glands and is localised mostly at the apical membrane [20]. Polymorphic expression of the MDR1 protein in the lung parenchyma may explain part of the differences in lung symptomatology observed in the CF patients carrying the same mutation [21]. A recent study [22] found an association between a low expression level of multidrug-resis-

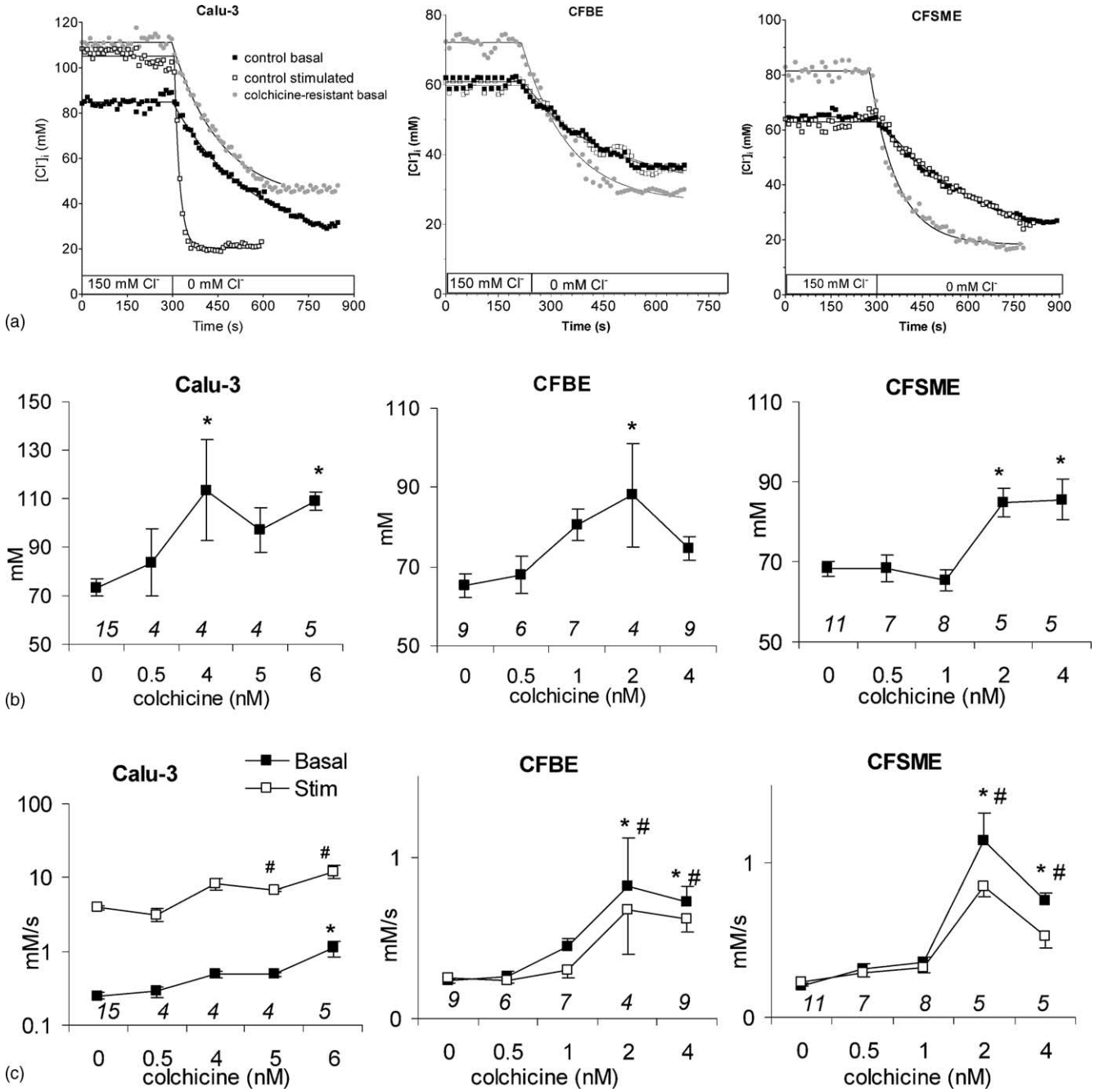

Fig. 4. The effect of colchicine on intracellular chloride concentration and efflux. (a) Representative recording of the intracellular chloride concentration during an efflux experiment. The rectangle above the time axis indicates the concentration of chloride in the extracellular solution bathing the cells. The basal trace was recorded in the absence of agonists, while the stimulated trace was obtained under the continuous presence of 5 μM forskolin and 100 μM IBMX. The trace marked colchicine-resistant was recorded in cells resistant to 4 nM colchicine. The symbols represent the experimental data, while the continuous lines are obtained by computer fitting to an exponential function. (b) The values of intracellular chloride concentration before the efflux under basal conditions in control and colchicine-resistant cells. (c) The values of chloride efflux rate under basal and cAMP-agonist stimulated conditions in control and colchicine-resistant cells. Values are indicated as mean ± standard error, and the number of experiments for a colchicine condition is displayed in *italics* above the horizontal axis. Significant difference from the control cells ($P < 0.05$, ANOVA followed by Dunnet's test) is indicated by symbol (*) in the case of basal efflux and ($^{\#}$) in the case of cAMP-stimulated efflux.

tance related proteins MRP1 and MRP5 and the severity of CF disease in 19 ΔF508 homozygous patients. This also correlated with a lower cAMP-independent Cl^- conductance of the respiratory epithelial cells, indicative of the important contribution of ABC proteins to the ion transport in epithelial cells [23].

The cells used for the present study had a low level of constitutive MDR expression. It has been well established that continuous exposure of cells to chemotherapeutic drugs such as the alkaloid colchicine induces the apparition of a ''multidrug resistance phenotype'' characterised by concomitant resistance to high concentration of un-related

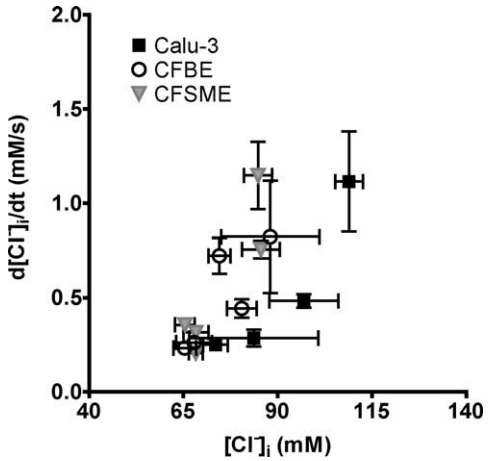

Fig. 5. The plot of the chloride efflux rate as a function of the intracellular chloride concentration under basal conditions. Data are shown as mean ± standard error on both axes.

drugs and at the molecular level, by the up-regulation and expression of the ABC protein family, including MDR1 and diverse MRP proteins [5]. Thus, the increase in MDR1 expression observed in the colchicine-resistant cells is not surprising and although not tested, we expect that even other proteins belonging to the same family to have been overexpressed.

CFTR and MDR1 genes have been shown to have complementary patterns of epithelial expression [24]. In colon epithelial cell lines, the gradual acquisition of resistance to colchicine was associated with a corresponding increased expression of MDR1 protein and a reversible decrease in the constitutive levels of CFTR, as determined by immunoblotting in human colon carcinoma cells [25]. Cao et al. observed that at high concentrations of the drug doxorubicin (128 μM), MDR1 overexpression was associated with a reduction in CFTR chloride channel activity, due to a promoter mediated decrease of CFTR mRNA and simultaneous increase of MDR1 mRNA [26]. Importantly, co-expression of MDR1 and CFTR had no effect on CFTR function. At nanomolar concentrations of colchicine, we found in our colchicine-resistant cells an increase in the relative amount of MDR protein, but without significant changes in the localisation of the CFTR protein. The differences observed in the CFTR staining of control and colchicine-resistant cells may be due to an increased expression of CFTR, or post-transcriptional events, without significant translocation of the defective protein to the cell membrane. The use of a monoclonal anti-CFTR antibody excludes the false-positive staining of other proteins in the cell and negative controls (where the first antibody was omitted) showed no staining (data not shown). It should be mentioned though, that the concentration of colchicine used in our study was 2 orders of magnitude smaller than that of colchicine used in the study of Breuer et al. [25], and thus maybe insufficient to down-regulate the constitutive levels of CFTR. In another study, selection of CFTR cells resistant to doxorubicin or vincristin produced increased levels of both MDR and CFTR mRNA [27]. A straightforward argument for the lack of negative effect of colchicine treatment on the CFTR function is the fact that in the Calu-3 cells (expressing wt-CFTR), the response to cAMP-elevating agents forskolin and IBMX was significantly increased in the colchicine-resistant cells compared to the control (Fig. 3c).

Several reports point towards involvement of the multidrug resistance phenotype in the regulation of ion transport in epithelial cells. The catalytic capacity of multidrug resistant cells exceeds the rate of passive diffusion of drugs in the concentration range 10^{-7} to 10^{2} μM, thus appearing to violate the laws of enzyme specificity, the coupling principle and the kinetics of active transport associated with the "ATP-driven pump model" function of the MDR1 protein [28]. The "altered partitioning model" proposes that MDR1 overexpression and the additional drug resistance mechanisms induced by chemotherapeutical drugs alter the retention of drugs indirectly, by modulating the intracellular pH, the volume, and/or the membrane potential [5,6]. Studies have shown that MDR expression dramatically inhibits the normal Cl^-/HCO_3^- exchange [9], can be a potent regulator of Cl^- conductance [29] or of the Cl^- gradient-stimulated H^+ transport [9]. Our observations of increased intracellular chloride concentration at higher colchicine concentration could be the result of changes in intracellular pH and membrane electric potential, in agreement with the "altered partitioning model". A small increase in the intracellular pH could explain the increased resting values of the intracellular chloride in the colchicine-resistant cells. Overexpression of the human MDR1 protein was shown to perturb the membrane potential and to clearly alkalinise the cytosol of eukariotic cells, in direct correlation to their multidrug resistance capacity [30]. Recently published evidence argues for an effect of human MDR1 protein on the membrane potential and ATP-regulated Cl^- conductance in proteoliposomes [12]. Another study argued that MDR1 overexpression did not change the membrane potential, but might perturb the intramembraneous potential [31]. Electrophysiological studies examining the putative ion transport via MDR1 protein reported unusual Cl^- transport in the cells with MDR1 overexpression compared to control cells [32,33]. Hainsworth et al. [34] observed that the cells expressing the MRP protein have a significantly increased activity of the hypotonicity-induced anion channel compared to control. MRP might conduct ATP and thus contribute to the autocrine regulation of the swelling-activating chloride channels [35]. We observed in the three colchicine-resistant cell types increased Cl^- efflux rates under basal conditions and a possible explanation might be the upregulation, among others, of the swelling-activated chloride channels.

Regardless of the mechanism, the long-term consequences of elevated intracellular Cl^- might be up-regulation of other outward Cl^- transport pathways and an increase in membrane permeability to Cl^-. It has been

suggested that colchicine treatment of cystic fibrosis patients would be beneficial [3,4]. In addition to its anti-inflammatory properties, colchicine would, as shown in the present study, increase chloride efflux from airway epithelial cells, thereby potentially alleviating the fluid transport defect. Colchicine does not exert its effect via CFTR, but via other chloride channels. Since the effect is likely to be due primarily to a change in the chloride gradient, it is unlikely that a specific chloride channel is involved, but the effect may be mediated by several channels.

Because MDR does not interfere with the function of CFTR, colchicine treatment could be instituted with small risks. Activation of alternative chloride channels is an accepted strategy for pharmacological treatment of cystic fibrosis [36,37]. There are indications that even short-term treatment with chemotherapeutical drugs may be effective [38]. However, the increase in the basal chloride efflux observed in our study was still far from the response of the wild-type CFTR carrying cells to cAMP agonists. Whether the effect of colchicine treatment would have clinical significance is therefore still an open question, which would need to be answered by clinical trials.

Acknowledgments

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References

[1] http://www.genet.sickkids.on.ca/cftr.
[2] Ben-Chetrit E, Levy M. Colchicine: 1998 update. Semin Arthritis Rheum 1998;28:48–59.
[3] Sermet-Gaudelus I, Stoven V, Annereau JP, Witko-Sarsat V, Reinert P, Guyot M. Interest of colchicine for the treatment of cystic fibrosis patients. Prelim Rep Mediat Inflamm 1999;8:13–5.
[4] Willemot JM, Sermet-Gaudelus I, Lenoir G. New therapeutic approaches to cystic fibrosis. Ann Pharm Fr 2003;61:253–8.
[5] Roepe PD. The role of the MDR protein in altered drug translocation across tumor cell membranes. Biochim Biophys Acta 1995;1241:385–405.
[6] Roepe PD, Wei LY, Hoffman MM, Fritz F. Altered drug translocation mediated by the MDR protein: direct, indirect, or both? J Bioenerg Biomembr 1996;28:541–55.
[7] Golstein PE, Boom A, van Geffel J, Jacobs P, Masereel B, Beauwens R. P-glycoprotein inhibition by glibenclamide and related compounds. Pflugers Arch 1999;437:652–60.
[8] Lallemand JY, Stoven V, Annereau JP, Boucher J, Blanquet S, Barthe J, et al. Induction by antitumoral drugs of proteins that functionally complement CFTR: a novel therapy for cystic fibrosis? Lancet 1997;350:711–2.
[9] Hoffman MM, Roepe PD. Analysis of ion transport perturbations caused by hu MDR1 protein overexpression. Biochemistry 1997;36:11153–68.
[10] Wei LY, Hoffman MM, Roepe PD. Altered pHi regulation in 3T3/CFTR clones and their chemotherapeutic drug-selected derivatives. Am J Physiol 1997;272:C1642–53.
[11] Gerard V, Rouzaire-Dubois B, Dilda P, Dubois JM. Alterations of ionic membrane permeabilities in multidrug-resistant neuroblastoma × glioma hybrid cells. J Exp Biol 1998;201:21–31.
[12] Howard EM, Roepe PD. Purified human MDR 1 modulates membrane potential in reconstituted proteoliposomes. Biochemistry 2003;42:3544–55.
[13] Rychlik B, Pulaski L, Sokal A, Soszynski M, Bartosz G. Transport of organic anions by multidrug resistance-associated protein in the erythrocyte. Acta Biochim Pol 2000;47:763–72.
[14] Krapf R, Berry CA, Verkman AS. Estimation of intracellular chloride activity in isolated perfused rabbit proximal convoluted tubules using a fluorescent indicator. Biophys J 1988;53:955–62.
[15] Chen PY, Illsley NP, Verkman AS. Renal brush-border chloride transport mechanisms characterized using a fluorescent indicator. Am J Physiol 1988;254:F114–20.
[16] Schinkel AH, Roelofs EM, Borst P. Characterization of the human MDR3 P-glycoprotein and its recognition by P-glycoprotein-specific monoclonal antibodies. Cancer Res 1991;51:2628–35.
[17] Putterman C, Ben-Chetrit E, Caraco Y, Levy M. Colchicine intoxication: clinical pharmacology, risk factors, features, and management. Semin Arthritis Rheum 1991;21:143–55.
[18] Taylor EW. The mechanism of colchicine inhibition of mitosis. J Cell Biol 1965;25:145–60.
[19] Deery WJ, Weisenberg RC. Kinetic and steady-state analysis of microtubules in the presence of colchicine. Biochemistry 1981;20:2316–24.
[20] Wioland MA, Fleury-Feith J, Corlieu P, Commo F, Monceaux G, Lacau-St-Guily J. CFTR, MDR1 and MRP1 immunolocalization in normal human nasal respiratory mucosa. J Histochem Cytochem 2000;48:1215–22.
[21] Johannesson M, Sandberg Nordqvist AC, Bogdanovic N, Hjelte L, Schalling M. Polymorphic expression of multidrug resistance mRNA in lung parenchyma of nonpregnant and pregnant rats: a comparison to cystic fibrosis mRNA expression. Biochem Biophys Res Commun 1997;239:606–11.
[22] Hurbain I, Sermet-Gaudelus I, Valle B, Feuillet MN, Lenoir G, Bernaudin JF, et al. Evaluation of MRP1-5 gene expression cystic fibrosis patients homozygous for the ΔF508 mutation. Pediatr Res 2003;54:627–34.
[23] Ito S. Is CFTR dysfunction in cystic fibrosis compensated by MRPs? Pediatr Res 2003;54:625–6.
[24] Trezise AE, Romano PR, Gill DR, Hyde SC, Sepulveda FV, Buchwald M, et al. The multidrug resistance and cystic fibrosis gene have complementary patterns of expression. EMBO J 1992;11:4291–303.
[25] Breuer W, Slotki IN, Ausiello DA, Cabantchik IZ. Induction of multidrug resistance downregulates the expression of CFTR in colon epithelial cells. Am J Physiol 1993;265:C1711–5.
[26] Cao L, Owsianik G, Jaspers M, Janssens A, Cuppens H, Cassiman JJ. Functional analysis of CFTR chloride channel activity in cells with elevated MDR1 expression. Biochem Biophys Res Commun 2003;304:248–52.
[27] Wei LY, Stutts MJ, Hoffman MM, Roepe PD. Overexpression of the cystic fibrosis transmembrane conductance regulator in NIH 3T3 cells lowers membrane potential and intracellular pH and confers a multidrug resistance phenotype. Biophys J 1995;69:883–95.
[28] Fritz F, Howard HM, Hoffman MM, Roepe PD. Evidence for altered ion transport in *Saccharomyces cerevisiae* overexpressing human MDR1 protein. Biochemistry 1999;38:4214–26.

[29] Hardy SP, Goodfellow HR, Valverde MA, Gill DR, Sepulveda V, Higgins CF. Protein kinase C-mediated phosphorylation of the human multidrug resistance P-glycoprotein regulates cell volume-activated chloride channels. EMBO J 1995;14:68–75.
[30] Hoffman MM, Wei LY, Roepe PD. Are altered pHi and membrane potential in hu MDR 1 transfectants sufficient to cause MDR protein-mediated multidrug resistance? J Gen Physiol 1996;108:295–313.
[31] Luker G, Flagg TP, Sha Q, Luker KE, Pica CM, Nichols CG, et al. MDR1 P-glycoprotein reduces influxes of substrates without affecting membrane potential. J Biol Chem 2001;276:49053–60.
[32] Bond TD, Higgins CF, Valverde MA. P-glycoprotein and swelling activated chloride channels. Methods Enzymol 1998;292:359–70.
[33] Idriss HT, Hannun YA, Boulpaep E, Basavappa S. Regulation of volume-activated chloride channels by P-glycoprotein: phosphorylation has the final say! J Physiol 2000;5243:629–36.
[34] Hainsworth AH, Henderson RM, Hickman ME, Hladky SB, Rowlands T, Twentyman PR, et al. Hypotonicity-induced anion fluxes in cells expressing the multidrug-resistance-associated protein, MRP. Pflugers Arch 1996;432:234–40.
[35] Darby M, Kuzmiski JB, Panenka W, Feighan D, MacVicar BA. ATP released from astrocytes during swelling activates chloride channels. J Neurophysiol 2003;89:1870–7.
[36] Roomans GM. Pharmacological treatment of the ion transport defect in cystic fibrosis. Exp Opin Invest Drugs 2001;10:1–19.
[37] Roomans GM. Pharmacological approaches to correcting the ion transport defect in cystic fibrosis. Am J Respir Med 2003;2: 513–31.
[38] Maitra R, Shaw CM, Stanton BA, Hamilton JW. Increased functional cell surface expression of CFTR and ΔF508-CFTR by the anthracycline doxorubicin. Am J Physiol 2001;280:C1031–7.