

Effects of cyclosporin at various concentrations on dexamethasone intracellular uptake in multidrug resistant cells

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Abstract

Background—The multidrug resistance phenomenon results from the expression of P-glycoprotein (P-gp), a drug-efflux pump. Corticosteroids are substrates for P-gp, whose function can be inhibited by cyclosporin. This study evaluates the ability of cyclosporin to modulate dexamethasone uptake in multidrug resistant cells.

Methods—The K 562 cell line, which does not express P-gp and a P-gp expressing clone, K562/ADM, were used. Cells were incubated with H3-dexamethasone in the absence or presence of cyclosporin at various concentrations. Then, cells were washed, lysed, and radioactivity was measured.

Results—The uptake of dexamethasone alone was higher in sensitive than in resistant cells. Addition of cyclosporin induced a dose dependent increase of dexamethasone uptake in resistant cells, whereas the drug did not influence dexamethasone uptake in parental cells.

Conclusion—Cyclosporin, at therapeutic concentrations induces a moderate, but significant increase in dexamethasone accumulation in multidrug resistant cells. Thus, cyclosporin might increase the intestinal absorption of corticosteroids or their accumulation in mononuclear cells, or both, thereby increasing their therapeutic efficacy.

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its potential role in some resistances to corticosteroids.

The observation that corticosteroids are substrates for P-gp, and that cyclosporin inhibits P-gp function suggest that cyclosporin might diminish the cellular efflux and increase the intracellular accumulation of corticosteroids in patients treated with both drugs, such as some rheumatoid arthritis (RA) patients. By the use of high concentrations of cyclosporin, we have previously shown that cyclosporin increases dexamethasone intracellular accumulation in cell lines expressing P-gp.⁷ Others have shown that cyclosporin and other immunosuppressants potentiate a dexamethasone mediated transcriptional response in LMCAT cells by inhibiting efflux of this corticosteroid.⁸ Such inhibition might⁸ or might not⁹ be mediated through P-gp blockade.⁸ However, the concentrations of cyclosporin used were much higher than clinically relevant concentrations. This study evaluates the effects of clinically relevant concentrations of cyclosporin on dexamethasone intracellular uptake in multidrug resistant cells.

Methods

We used a human leukaemic resistant cell line (K562/ADM) derived from the parental K562 cell line by continuous exposure to increasing concentrations of doxorubicin. These clones were kindly provided by Dr Tsuoro (Cancer Chemotherapy Center, Tokyo, Japan). The MDR phenotype is not expressed by K562 but is overexpressed in K562/ADM. The expression of P-gp protein in K562 and K562/ADM cells was checked by cytofluorimetry using MRK16, a specific mouse monoclonal antibody (Immunotech, Marseille, France). Cells were grown in suspension in RPMI 1640 medium (Gibco, Paisley, UK) supplemented with 2 mM L-glutamine and 10 % fetal bovine serum at 37°C in a humidified atmosphere (5% carbon dioxide and 95% air).

K 562 and K562/ADM cells were seeded in 96 well flat bottom microtitre plates (2.10⁵ cells per well) and were incubated for four hours at 37°C in culture medium with 50 µg/ml dexamethasone (99.92% dexamethasone and 0.08% 1, 2, 4,6, 7-(³H)-dexamethasone, Amersham; Les Ulis; France), in the absence or in the presence of cyclosporin A at various concentrations (0.05, 0.15, 0.6, 1 and 10 µmol/l). Each determination was made in triplicate. The dexamethasone concentration was high, at least with respect to pharmacological serum concentrations. We used such a concentration

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Primary or acquired resistance of cancer to cytotoxic drugs is part of a larger phenomenon described as multidrug resistance (MDR).^{1,2} MDR results from the expression of a 170 kDa membrane glycoprotein (P-gp), which acts as a drug-efflux pump.^{1,2} P-gp or its encoding gene (*mdr 1*) has been detected in many human cancers, in either untreated or previously treated tumours.³ Reversal of drug resistance has been obtained by different agents, which usually act as competitive inhibitors. These so called resistance modifying agents include verapamil, quinine derived compounds and cyclosporin.⁴

Several observations suggest that some corticosteroids could be physiological substrates for P-gp. These compounds have been proposed to interfere with the MDR system by either modulating *mdr 1* gene expression or acting as competitive inhibitors for P-gp drug transport function.⁵⁻⁷ The potential interactions between corticosteroids and the MDR system raise interesting questions, including

because it allowed us to measure easier a pharmacological effect. After incubation, the cells were washed three times with ice cold phosphate buffered saline, lysed in NaOH 1N, then transferred into counting vials with 3 ml scintillation liquid (LKB, Stockholm, Sweden). Radioactivity was measured (240 seconds per vial) in a β -scintillation counter (LKB 1214, Rackbeta, Stockholm, Sweden). Three independent experiments were performed to test the intracellular uptake of dexamethasone. Results were primarily expressed as the mean (SD) radioactivity (cpm) of nine wells (three independent experiments, three wells per experiment). Then, to express results in absolute unit (pmol of ^3H -dexamethasone per cell), 200 μl of culture medium containing various amount of ^3H -dexamethasone (0.776; 7.76; 77.6 and 776 pg) were transferred in counting vials with 3 ml scintillation liquid, and radioactivity was measured.

The Mann-Whitney test was performed to compare the intracellular uptake of dexamethasone between sensitive and resistant cells with various concentrations of cyclosporin A. The uptake of dexamethasone in sensitive cells at various concentrations of cyclosporin was compared with the uptake without cyclosporin (Mann-Whitney). Finally, the uptake of dexamethasone in resistant cells at various concentrations of cyclosporin was compared with the uptake without cyclosporin (Mann-Whitney). Statistical significance was defined as $p < 0.05$.

Results

As figure 1 shows, the intracellular uptake of dexamethasone alone was four to five times higher in P-gp-negative K 562 cells than in P-gp-positive K562/ADM cells ($p < 0.0005$). The addition of cyclosporin did not affect the dexamethasone uptake in sensitive cells ($p < 0.1$, 0.4, 0.2, 0.9 and 0.9 at 0.05, 0.15, 0.6, 1 and 10 $\mu\text{mol/l}$ respectively). In contrast, cyclosporin induced a dose dependent increase of dexamethasone uptake in resistant cells (+ 31.9%, $p < 0.04$; + 39.9%, $p < 0.04$; + 62%, $p < 0.005$; + 297.3%, $p < 0.0005$ and + 354.6%, $p < 0.0005$ at 0.05, 0.15, 0.6, 1 and 10 $\mu\text{mol/l}$ respectively).

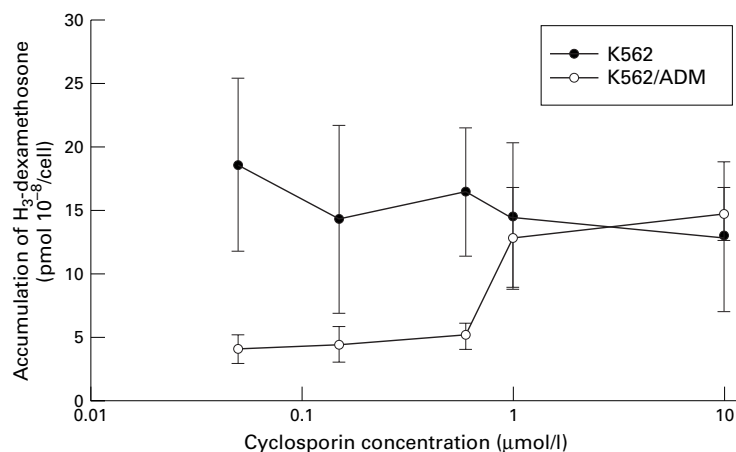


Figure 1 Intracellular uptake of dexamethasone in sensitive (K 562) and resistant (K 562/ADM) cells at various concentrations of cyclosporin.

Discussion

Although most studies regarding the MDR system have been conducted in oncology, a few works have been published in RA patients. Both the *mdr-1* gene and the P-gp were shown to be expressed in the synovial tissue of patients with RA.¹⁰ We observed that the percentage of peripheral blood lymphocytes expressing P-gp was higher in RA patients treated with prednisolone than in RA patients not exposed to corticosteroids.¹¹ Matsubara analysed the expression of *mdr-1* gene in peripheral blood mononuclear cells of RA patients before starting gold treatment.¹² After a few months of treatment, the improvement ratio was higher in patients with the lower expression of *mdr-1* gene before treatment. Consequently, it was proposed that P-gp expression might play a part in the clinical resistance of some RA to drugs,^{10 11 13} especially corticosteroids.¹¹

In RA patients, cyclosporin has been used as a disease modifying anti-rheumatic drug for several years. The efficacy of cyclosporin presumably relies on a suppression of T cell mediated autoimmunity. However, the efficacy might rely on additional modes of action. A number of RA patients treated with cyclosporin are co-treated with corticosteroids. These drugs are substrates for P-gp, whose function is inhibited by cyclosporin. Consequently, an additional mechanism of action of cyclosporin might be an enhancement of the intracellular accumulation of corticosteroids. At least two mechanisms could account for the ability of cyclosporin to modulate cellular accumulation of corticosteroids. Firstly, cyclosporin could inhibit drug efflux by P-gp expressed by human mononuclear cells. These cells have been shown to express P-gp.¹³ Although the percentage of peripheral blood lymphocytes expressing P-gp does not increase in RA patients compared with controls (except for patients treated with prednisolone),¹¹ the activity of the protein was shown to be higher in RA patients when compared with controls.¹⁴ In these patients, cyclosporin might reduce the mononuclear cell extrusion of corticosteroids, leading to an increased efficacy of these drugs.

Secondly, cyclosporin could modulate the activity of P-gp in the intestine. P-gp is located within the brush border on the apical surface of the enterocytes, where it pumps P-gp substrates from the enterocyte back into the intestinal lumen.^{15 16} Cyclosporin might reduce the transport of corticosteroids into the intestinal lumen, leading to an increase in intestinal absorption of corticosteroids.

The resistance modifying agents are sometimes difficult to use, in that they might have toxic effects at concentrations needed to block drug efflux by P-gp. Cyclosporin increases dexamethasone accumulation in resistant cells in a dose dependent manner, reaching a plateau phase at 1 $\mu\text{mol/l}$. This concentration is not obtained in RA patients. However, at clinically relevant concentrations, cyclosporin induced a moderate, but significant increase in intracellular dexamethasone accumulation. A number of RA patients treated with cyclosporin are co-treated with corticosteroids.

Thus, in these patients, an additional mechanism of action of cyclosporin might be a moderate increase in intracellular uptake of these drugs. In resistant patients, it could be of interest to determine dexamethasone accumulation in vitro in PBMC in the presence and absence of cyclosporin, and to determine serum corticosteroid concentrations with or without co-administration of cyclosporin.

A drawback of this work is that dexamethasone is not usually used in RA. Our results might not be observed with more commonly used corticosteroids, such as prednisolone or prednisone (which is converted to prednisolone in vivo). Some authors have evaluated the resistance to various corticosteroids of a P-gp expressing variant cell line.⁶ The corticosteroids could be divided in three classes: class I to which the variant had no increased resistance, class II to which the variant had modest resistance, and class III to which the variant acquired considerable resistance. Dexamethasone and prednisolone both belonged to the class III, with comparable resistance levels. Consequently, this suggests that our results should be observed using prednisolone instead of dexamethasone.

In conclusion, cyclosporin, at concentrations used in RA patients, induces a moderate, but significant increase in dexamethasone accumulation in cells expressing the MDR phenotype. Thus, besides its well characterised immunomodulating activity, cyclosporin might increase the accumulation of corticosteroids in mononuclear cells or their intestinal absorption, or both, thereby increasing their therapeutic efficacy.

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