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Ciprofloxacin and Doxorubicin are substrates of different Multidrug Resistance-related Proteins efflux transporters in J774 macrophages

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ABSTRACT

Objectives: Ciprofloxacin (CIP) and doxorubicin (DOX) are both substrates of the Multidrug Resistance-related Proteins efflux transporters (MRP), a subfamily of the ATP-Binding Cassette transporters (ABC) in eukaryotic cells. This contributes to a reduction of their cellular accumulation, and, therefore, of their intracellular activity (Int J Cancer. 2001;93:107-13; J Antimicrob Chemother. 2003;51:1167-73). Our objective was to determine whether CIP and DOX could share the same MRP transporter in macrophages since this could be the basis of potential, unanticipated drug interactions in cancer patients receiving both drugs either simultaneously (by direct competition for transport) or in succession (trough drug-induced overexpression of the transporter).

Methods: We used J774 macrophages, a cell line that spontaneously expel CIP (Antimicrob Agents Chemother. 2004;48:2673-82; wild-type cells) and a cell line derived thereof by continuous exposure to CIP (CIP-resistant cells) and which overexpresses the corresponding MRP transporter (Antimicrob Agents Chemother. 2006;50:1689-95). Cells were incubated with CIP or DOX alone, or in competition with each other, or in the presence of probenecid (PB), a non-specific inhibitor of MRP transporters. Cell-associated CIP and DOX were measured by fluorimetry and their accumulation recorded as cellular to extracellular concentration ratios (Cc/Ce).

Results: In wild-type cells, (i) PB increased both CIP and DOX Cc/Ce but not to the same extent; (ii) excess of CIP but not of DOX caused an increase in CIP Cc/Ce; (iii) excess of DOX had no effect on CIP and only a marginal effect on DOX Cc/Ce. In CIP-resistant cells, accumulation of CIP was markedly reduced compared to wild-type cells (and only partially restored by PB), but not that of DOX (with PB exerting a similar effect to that observed in wild-type cells).

Condition	Cc/Ce (% control in wild-type cells)			
	wild-type cells		CIP-resistant cells	
	CIP	DOX	CIP	DOX
control (drug conc. 50 µM)	100 ± 1	100 ± 8	10 ± 2 *	98 ± 10 ns
+ PB (5 mM)	335 ± 24 *	178 ± 41 *	225 ± 41 *	195 ± 16 ns
+ DOX (total conc. 75 µM *)	101 ± 2 ns	135 ± 16 *		
+ CIP (total conc. 500 µM *)	494 ± 43 *	105 ± 6 ns		

* highest testable conc. (limit of solubility)

Statistical analysis: (i) wild-type cells: differences with control values; (ii) CIP-resistant cells: differences with the corresponding values in wild-type cells; * $p < 0.05$; ns non significant.

Conclusions: **CIP and DOX are substrates of distinct MRP efflux transporters in J774 cells. Drug interactions related to competition for transport or overexpression of CIP transporter are, therefore, unlikely.**

INTRODUCTION

- Overexpression of active Multidrug Resistance Proteins like P-gp or MRPs is a well known mechanism of resistance to anticancer agents. These efflux pumps, however, are characterized by a broad substrate specificity and are capable of transporting out of eukaryotic cells other drugs like antibiotics (1).
- Recent work of our laboratory has shown that ciprofloxacin is susbtrate of the an efflux pump of the MRP family in J774 macrophages and that the chronical exposure of macrophages to high ciprofloxacin concentrations can select ciprofloxacin-resistant cells, which accumulate reduced amounts of drug and are therefore able to survive in the presence of high ciprofloxacin concentrations (2). 9 MRP have been described so far, the ciprofloxacin transporter has not yet been identified.
- Doxorubicin is known as a substrate of MRP transporters, and of MRP-1 in particular (3).
- Competiton for a same efflux transporter can be considered as a new mechanism of drug interactions, which can contribute to modify the cellular pharmacokinetics of drugs substrates/inhibitors of the same efflux transporter (4).

AIMS OF THE STUDY

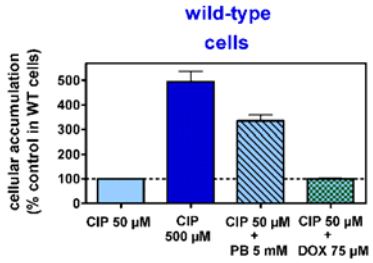
- To compare the cellular accumulation of ciprofloxacin and doxorubicin in wild-type macrophages and in ciprofloxacin-resistant macrophages, which overexpress the ciprofloxacin MRP transporter.
- To examine whether these two drugs compete for a same MRP transporter in these two cell lines.

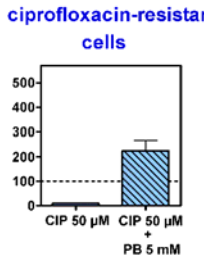
RESULTS

CIPROFLOXACIN ACCUMULATION

• in wild-type cells
(expressing efflux transporters at a basal level):
- is higher in cells exposed to high CIP concentrations (suggesting that CIP inhibits its own efflux)
- is increased by probenecid, a broad spectrum inhibitor of organic anions and MRP transporters
- is not modified in the presence of DOX

• in ciprofloxacin-resistant cells
(overexpressing the MRP transporter responsible for CIP efflux):
- is considerably lower than in wild-type cells
- is increased in the presence of probenecid, but to a lower extent than in wild-type cells

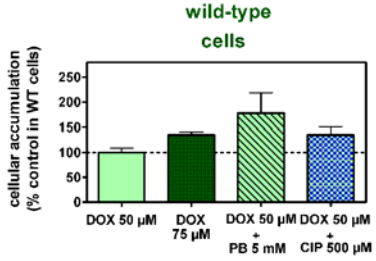


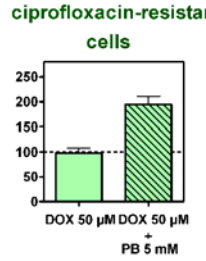


DOXORUBICIN ACCUMULATION

• in wild-type cells :
- is slightly increased in cells exposed to high DOX concentrations
- is increased by probenecid
- is marginally increased in the presence of high CIP concentrations

• in ciprofloxacin-resistant cells
- is similar than in wild-type cells
- is increased in the presence of probenecid, but to the same level than in wild-type cells





METHODS

- **Cell lines**: we used in parallel wild-type J774 mouse macrophages and ciprofloxacin-resistant J774 macrophages. These cells were obtained by chronical exposure of J774 wild-type cells to high concentrations of ciprofloxacin (2).
- **Cellular accumulation studies:** cells were incubated with each drug alone or combined with the other drug or with probenecid during 2h, washed 3 times in ice-cold PBS and collected for assay of drug and protein content (2).
- **Drug assays:** both drugs were assayed by fluorimetry (2,5), using the following conditions
CIP: λexc: 275nm; λem: 450nm; Glycine-HCl 0.1 M pH 3
DOX: λexc: 475 nm; λem: 540 nm; EtOH:HCl 3 N 1:1

CONCLUSIONS

- CIP and DOX are both substrates for a probenecid-inhibitable transporter in J774 macrophages.
- DOX is not substrate for the CIP transporter, since its accumulation is not reduced in CIP-resistant macrophages.
- CIP and DOX inhibit their own efflux, causing a higher accumulation in cells exposed to higher concentrations. In contrast, they do not markedly influence the accumulation of the other drug in wild-type cells, suggesting
  - that their probably do not share the same transporter
  - that drug interactions mediated by competition for the same transporter are unlikely.

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