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Competition between Ciprofloxacin and Non-Steroidal Anti-Inflammatory Drugs for Efflux Transporters in J774 Macrophages

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Background : CIP is substrate for an MRP efflux transporter (AAC 2004,48:2673) that reduces its accumulation and intracellular activity in J774 macrophages. Since MRPs recognize anionic amphiphilic compounds, we have examined here potential competition between CIP and NSAIDs (indomethacin [IND], diclofenac [DIC]), all these drugs possessing a free carboxyl function and an hydrophobic nucleus.
Methods : We used wild-type (WT) and CIP-resistant (overexpressing the CIP transporter [AAC 2006, 50:1689]) macrophages. Cells were incubated with CIP or NSAID alone, or in competition with each other, or in the presence of gemfibrozil (GEM), a non-specific inhibitor of MRPs. Cellular concentration of drugs was measured by HPLC.
Results : the table shows the drug cellular accumulation (cell. to extracell. conc. ratio) measured after 6 h of incubation.

Condition	accumulation					
	WT cells			CIP-resistant cells		
	CIP	IND	DIC	CIP	IND	DIC
drug alone (50 µM)	6.2 ± 0.1 (a)	7.8 ± 1.2 (a)	3.1 ± 0.4 (a)	1.8 ± 0.8 (d)	1.4 ± 1.9 (h)	4.9 ± 0.6 (j)
+ GEM (500 µM)	20.9 ± 0.7 (b)	9.3 ± 0.3 (a)	9.2 ± 0.2 (b)	17.4 ± 0.6 (e)	8.2 ± 0.7 (a)	14.7 ± 0.7 (h)
+ IND (300 µM)	20.3 ± 0.1 (b)	na	na	14.3 ± 1.5 (f)	na	na
+ DIC (315 µM)	17.6 ± 1.7 (c)	na	na	3.2 ± 0.2 (g)	na	na
+ CIP (540 µM)	na	6.9 ± 0.1 (a)	3.9 ± 0.6 (a)	na	6.3 ± 2.1 (a)	5.0 ± 0.3 (i)

na: not applicable
Stat. analysis: Figures with another letter than (a) are significantly different from the corresponding "drug alone" value in WT cells and from values with other letters in the same column (ANOVA; p < 0.05)

CIP and IND accumulation was markedly reduced in CIP-resistant cells. IND and DIC were as efficient as GEM to increase CIP accumulation in WT cells, but DIC was much less effective in CIP-resistant cells. GEM increased IND and DIC accumulation in both cell types. CIP increased IND accumulation in CIP-resistant cells only.
Conclusions: CIP, IND and DIC are all substrates of GEM-inhibitable transporter(s). Cross-competition experiments and accumulation levels in CIP-resistant cells suggest that CIP and IND share a same transporter, while DIC is only a poor inhibitor of this transporter. These data (a) suggest that the CIP transporter is truly a multidrug transporter; (b) highlight potential drug interactions between CIP and pharmacologically-unrelated agents due to competition for transport.

INTRODUCTION

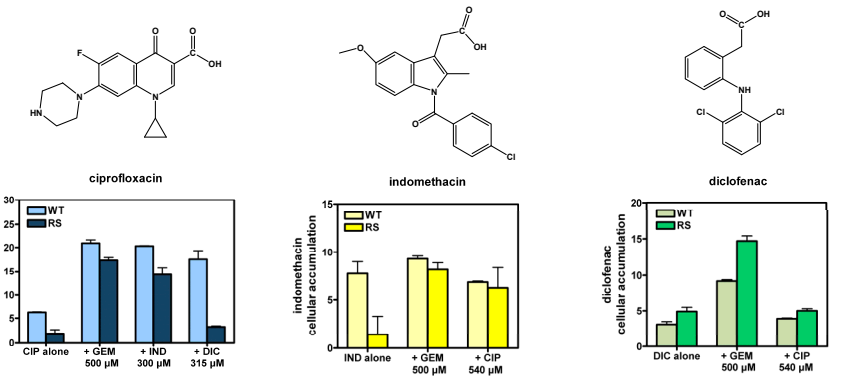
- Active efflux is a now recognized as a major determinant in the pharmacokinetic profile of drugs. Efflux pumps can indeed modulate the absorption, distribution or elimination of the drugs, as well as their capacity to accumulate within eucaryotic cells [1].
- Previous work of our laboratory has shown that CIP is substrate for an ABC transporter belonging to the MRP family in J774 macrophages [2] and that chronical exposure of these cells to increasing concentrations of CIP allows for the selection of resistant macrophages, which accumulate CIP to a much lower extent, suggestive of increased efflux capabilities [3].
- NSAIDs share with CIP some physicochemical properties (carboxylic function; aromatic rings) and have been described as MRP inhibitors [4].
- Competition for a same transporter may constitute a mechanism of drug interactions, which is still ill-explored.

AIM OF THE STUDY

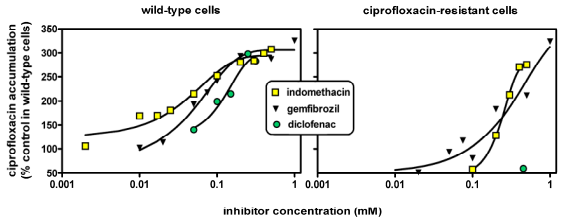
To examine whether ciprofloxacin and NSAIDs can compete for a same transporter in J774 macrophages. To this effect, we compared

- the effect of a known inhibitor of CIP efflux (gemfibrozil) with that of two NSAIDs (indomethacin, diclofenac) on ciprofloxacin accumulation in WT macrophages and in CIP-resistant macrophages;
- the effect of gemfibrozil and of ciprofloxacin on NSAID accumulation in the two cell types.

RESULTS



Cellular accumulation of ciprofloxacin (CIP; left), indomethacin (IND; middle), and diclofenac (DIC; right) in wild-type macrophages (WT; clear color) or in ciprofloxacin-resistant macrophages (RS; dark color) incubated during 6 h in the presence of the drug alone at 50 µM or combined with gemfibrozil (GEM) or one of the other drugs under investigation at the indicated concentration. Data are means ± SD (n= 3).



Influence of increasing concentrations of gemfibrozil (GEM), indomethacin (IND), or diclofenac (DIC) on the cellular accumulation of ciprofloxacin in wild-type macrophages (left) or in ciprofloxacin-resistant macrophages (right) measured after 6 h of incubation.
Data are means ± SD (n= 3).

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MATERIALS & METHODS

- **cells:** we used in parallel wild-type (WT) and CIP-resistant (RS) J774 macrophages. The latter were obtained by chronical exposure of WT macrophages to increasing concentrations of CIP [3].
- **cell treatment:** both cell types were exposed during 6 h to either CIP, IND, or DIC alone (at an extracellular concentration of 50 µM), or combined with GEM (as a known inhibitor of CIP efflux), or one of the other drugs under investigation (maximal extracellular concentration of each drug corresponding to its liit of solubility in the culture medium).
- **drug assay:** All drugs were assayed by HPLC (adaptation of methods published for fluoroquinolones [5], IND [6] or DIC [7]). Drug cellular concentration was expressed by reference to the protein content and accumulation factors were calculated using a conversion factor of 3.08 µl/mg prot, as determined previously for J774 macrophages [2].

DATA DESCRIPTION

ACCUMULATION DATA

Ciprofloxacin:
- CIP accumulation is reduced in CIP-resistant (RS) cells.
- In WT cells, CIP accumulation is increased to a same level by GEM, IND and DIC.
- In RS cells, only GEM and IND are able to increase CIP accumulation to a same level as in WT cells.

Indomethacin:
- IND accumulation is reduced in CIP-resistant (RS) cells.
- In WT cells, neither GEM, nor CIP affected IND accumulation.
- In RS cells, both GEM and CIP increase IND accumulation to the level measured in WT cells.

Diclofenac:
- DIC accumulation is similar in WT and in CIP-resistant (RS) cells.
- In WT cells, DIC accumulation is increased by GEM but not by CIP.
- In RS cells, GEM and CIP have similar effects as in WT cells.

DOSE - EFFECTS RELATIONSHIPS

The table shows the calculated concentration of each competitor needed to reach 50 % of the maximal ciprofloxacin accumulation in the corres-ponding cell line.

competitor	concentration causing a 50 % increase in CIP accumulation	
	WT cells	RS cells
GEM	63 µM	450 µM
IND	35 µM	315 µM
DIC	100 µM	>1000 µM

CONCLUSIONS

- Indomethacin and ciprofloxacin are probably substrates for a same transporter in J774 macrophages, since both of them accumulate to a lower level in CIP-resistant cells and increase the accumulation of the other drug.
- Diclofenac is a weak inhibitor, but not a substrate of ciprofloxacin transporter; it is probably rather substrate of another gemfibrozil-inhibitable transporter.
- Our data suggest that the CIP transporter is truly a "multidrug" transporter; they also highlight potential drug interactions between CIP and pharmacologically-unrelated agents due to competition for transport.