Fluoroquinolone recognition by prokaryotic *S. aureus* NorA and eukaryotic murine Mrp4 efflux transporters: a combined experimental and structural study

J. Dupont,[1] C.M. Vallet,[2] P.M. Tulkens,[2] M. Prévost,[1] F. Van Bambeke[2]

[1] Structure et fonction des membranes biologiques, Université libre de Bruxelles,
[2] Pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Brussels, Belgium

Mailing address:
Françoise Van Bambeke
av. Mounier 73, B1.73.05
1200 Brussels – Belgium
francoise.vanbambeke@uclouvain.be

22nd ECCMID 2012 London, United Kingdom 31 MAR-2 APR

P-1279

INTRODUCTION

Active efflux is a general mean of protection of cells against invasion by foreign and / or potentially toxic substances, including drugs like antibiotics (Biochem Pharmacol 2000, 60:457-70).

Antibiotic efflux confers resistance in bacteria and modulates accumulation and transport in eukaryotic cells.

AIM OF THE STUDY

To compare the *S. aureus* NorA (Major Facilitator Superfamily) and the mouse macrophage Mrp4 (ATP Binding Cassette superfamily) transporters with respect to recognition of fluoroquinolones (FQ).

METHODS

Physico-chemical properties: calculated with the Reaxys web-based software [http://www.reaxys.com]).

Efflux by NorA: ratio of MIC observed with the SA-1 *S. aureus* strain overexpressing NorA (AAC 2006, 50:1931-6) to that of the isogenic strain ATCC 25923 (the latter in the presence of reserpine [10 mg/L] to inhibit basal efflux by constitutively expressed NorA).

Efflux by Mrp4: ratio of accumulation observed in J774 macrophages overexpressing this transporter (AAC 2009, 53:2410-6) to that observed in wild-type cells (the latter with the Mrp4 inhibitor gemfibrozil [0.5 mM] to inhibit basal efflux by constitutively expressed Mrp4).

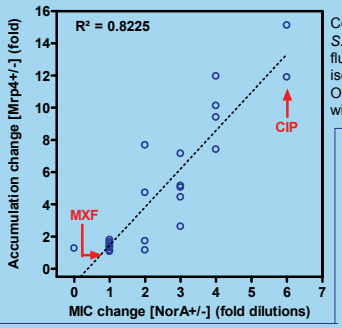
Conformational analysis: 3D models constructed by homology modeling using the Modeller program based on crystal structures of the prokaryotic lactose permease (LacY) for NorA (PDB code: 2CFQ) and of the eukaryotic P-glycoprotein for Mrp4 (PDB code: 3G5U); docking was perfomed using the induced fit procedure of the Schrodinger program. Putative binding site of NorA defined as that of the lactose analog in LacY complex structure and of Mrp4 identified using mutagenesis data (Mol. Pharmacol. 2008, 74:964-71).

CONCLUSIONS

- No simple correlation could be observed between susceptibility to efflux and fluoroquinolone physicochemical properties.
- Although phylogenetically very different from each other, NorA and Mrp4 show similar recognition properties for fluoroquinolones.
- Fluoroquinolones that undergo little efflux actually feature more numerous interactions in the binding sites. This suggests that the lack of transport is not due to poor recognition but rather to the inability of tightly bound drugs to progress for efflux within the transporter.

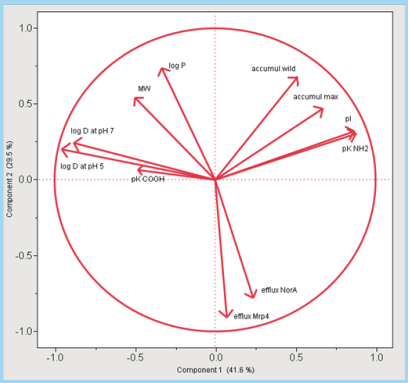
RESULTS

Correlation between efflux by NorA and by Mrp4



Correlation between the susceptibility to efflux by the prokaryotic efflux pump NorA of *S. aureus* and the eukaryotic efflux pump Mrp4 from mouse macrophages, for 24 fluoroquinolones. Abscissa: ratio of MICs between SA-1 (overexpressing NorA) and isogenic ATCC25923 in the presence of 10 mg/L reserpine (inhibition of basal efflux); Ordinate: ratio of accumulation between J774 macrophages overexpressing Mrp4 and wild-type cells in h presence of 500 µM gemfibrozil (inhibition of basal efflux).

Susceptibility to efflux vs. physicochemical properties



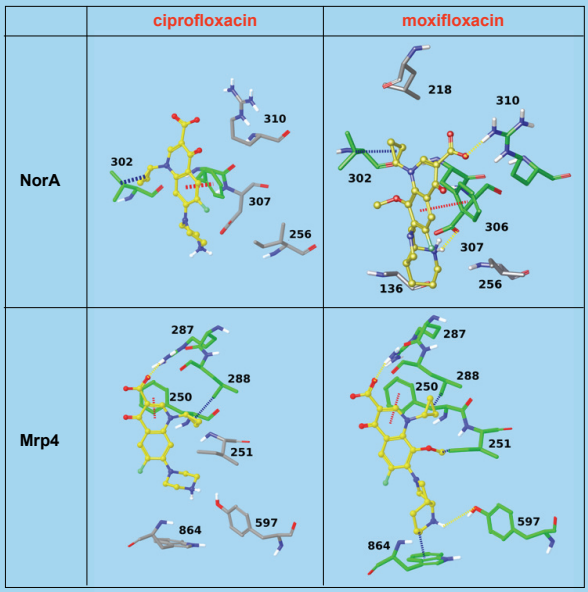
Principal component analysis of the correlations between biophysical properties of fluoroquinolones (ionization [pK COOH, pK NH2]; lipophilicity [log P; log D at pH 5 and 7]; molecular weight [MW]; and isoelectric point [pI]), accumulation in wild type cells (accumul wild), accumulation in cells in presence of gemfibrozil (accumul max), efflux related to activity of the bacterial transporter NorA (efflux NorA) and efflux related to the eukaryotic transporter Mrp4 (efflux Mrp4). The closeness of the arrows denotes the degree of correlation and their length the corresponding significance. This tool, using orthogonal linear transformation, is mostly used in exploratory data analysis and for making predictive models (Data analysis and graph generation with JMP Software version 9.0.3, SAS Institute Inc., Cary, NC).

Conformational analysis of ciprofloxacin in the binding pocket of NorA and Mrp4 and analysis of interactions with residues

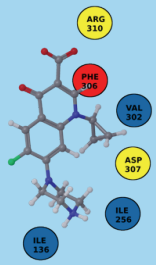
Left : Docked positions of ciprofloxacin and moxifloxacin in the putative binding sites of a modelled 3D structure of NorA and Mrp4. The ligands are depicted as ball-and-sticks and the interacting residues as sticks.

Middle : The table lists the interactions made by 9 different fluoroquinolones with either NorA or Mrp4. The color code is as follows: yellow for ionic interactions and hydrogen bonds, blue for hydrophobic interactions, and red for π–π interactions.

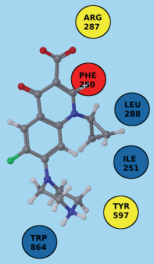
Right : Schematic representation of the residues interacting with fluoroquinolone in NorA (top) and Mrp4 (bottom)



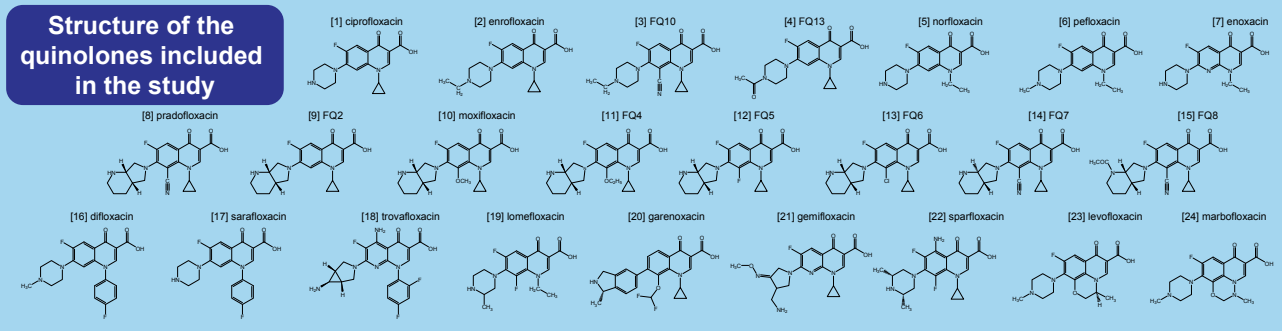
NorA	310	306	307	302	256	136	Nb interactions
[1] CIP							2
[5] NOR							2
[4] FQ13							2
[8] PRA							3
[14] FQ7							3
[9] FQ2							3
[6] PEF							4
[2] ENR							4
[10] MXF							4



Mrp4	287	288	250	251	597	864	Nb interactions
[1] CIP							3
[5] NOR							3
[4] FQ13							3
[8] PRA							4
[14] FQ7							4
[9] FQ2							5
[6] PEF							5
[2] ENR							5
[10] MXF							6



Structure of the quinolones included in the study



This poster will be made available for download after the meeting: http://www.facm.ucl.ac.be/posters.htm

Acknowledgments:
We thank Dr H. G. Wetzstein (Bayer, Leverkusen, Germany) for the kind gift of some of these molecules.