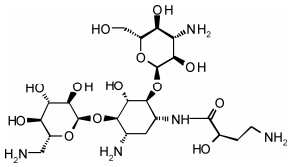
Reverse Iontophoresis of Amikacin: Contributions of Electroosmosis and Electrorepulsion

Fabio Marra, Giulianna Longobucco, Sara Nicoli, Patrizia Santi
Department of Pharmacy, University of Parma, Italy.

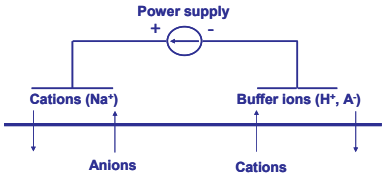
INTRODUCTION

Amikacin (AK) is an aminoglycosidic antibiotic active against most of gram-negative bacteria. Systemic aminoglycosides can produce hearing loss and balance difficulties and toxicity towards renal function. Therefore a careful monitoring of plasmatic aminoglycosides concentrations is required.



MW: 585.6
pKa: 8.4, 6.7, 9.7, 8.4

OBJECTIVES



The aim of this work was to explore the possibility to use reverse iontophoresis as non-invasive method to monitor AK plasmatic concentration. Acetaminophen (AM) was used as neutral marker, to quantify elecroosmotic flow contribution.

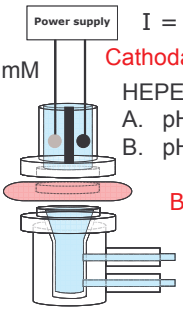
METHODOLOGY

Reverse iontophoresis :

The experiments lasted 2 h and 200 µl were withdrawn every 30 minutes from anodal and cathodal compartment, filled with the extraction solution.

Anodal compartment
HEPES 25 mM; NaCl 133 mM
A. pH 4.0
B. pH 8.0

Membrane
Rabbit ear skin



I = 0.5 mA/cm²

Cathodal compartment
HEPES 25 mM; NaCl 133 mM
A. pH 4.0
B. pH 8.0

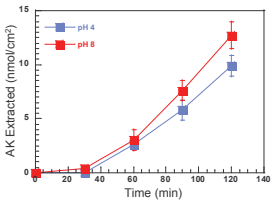
Bathing solution
HEPES 25 mM; NaCl 133 mM; pH 7.4
AK bisulphate 200 µM; AM 1 mM.

AK HPLC analysis

Samples derivatizated with 1-fluore-2,4-dinitrobenzene (FDNB) to be UV-Vis detectable:
- 100 µl of the sample mixed with 300 µl of methanol, 40 µl of NaOH 0.05 and 50 µl of methanolic solution of FDNB (180 mg/ml).
- Mixture heated at 90°C for 10 min.
- Column: 10 µm µBondapack® (300X4.6 mm) thermostated at 45°C
- Mobile phase: acetonitrile: water 47:53 (v/v)+ 0.1% acetic acid
- Flow: 1 ml/min
- Spectrophotometric detection: 365 nm

RESULTS AND DISCUSSIONS

AK cathodal extraction

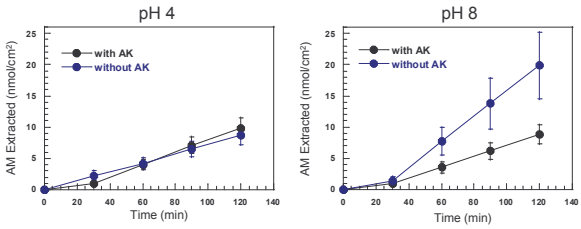


The extraction of AK was independent of pH and always in the anode-to-cathode direction, in agreement with the positive charge of the drug.

Total amount of AM and AK extracted after 2 h of reverse iontophoresis (average±sd)

pH of extraction solution	Polarity of reverse iontophoresis	AM Extracted (nmol/cm²)		AK Extracted (nmol/cm²)
		With AK	*Without AK*	
4.0	Anodal	2.91±0.36	2.91±0.19	-
	Cathodal	9.86±1.56	8.67±1.53	9.89±0.96
8.0	Anodal	1.00±0.18	1.99±0.77	-
	Cathodal	8.79±1.52	19.86±4.27	12.73±1.26

AM cathodal extraction

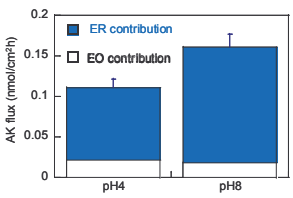


The presence of AK in the bathing solution did not modify AM extraction at pH 4.0. AM extraction was reduced in presence of AK at pH 8.0.

AK can alter permselectivity properties of the skin, reducing electroosmotic flow.

Contribution on AK cathodal extraction (average± sd)

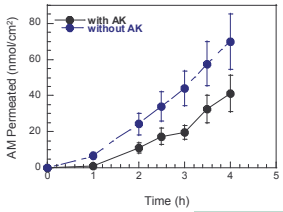
$J_{TOT} = J_{ER} + J_{EO}$



pH	J_{TOT} AK*10 (nmol cm^{-2} h^{-1})	J_{ER} AK*10 (nmol cm^{-2} h^{-1})	J_{EO} AK (%)
4.0	1.11±0.33	0.89±0.29	19.38
8.0	1.61±0.39	1.43±0.41	11.03

J_{TOT} = Total Flux
J_{ER} = Electrorepulsive Flow
J_{EO} = Electroosmotic Flow

AM direct anodal iontophoresis



Direct anodal iontophoresis of AM (donor concentration 10 mM) with AK 200 µM or without AK confirms the interaction of the positively charged AK with the skin.

Reduction of electroosmotic flow

CONCLUSIONS

- AK can be extracted across the skin in vitro by reverse iontophoresis.
- Cathodal extraction is higher than anodal, since AK is positively charged.
- Electrorepulsion is the main iontophoretic mechanism involved.
- AK alters skin permselectivity at pH 8, interacting with skin and decreasing elecroosmotic flow.

REFERENCES

1. S. Nicoli and P. Santi. Assay of amikacin in the skin by high-performance liquid chromatography. *J Pharm Biomed Anal* (in press).
2. S. Nicoli, M. Cappellazzi, P. Colombo, and P. Santi. Characterization of the permselective properties of rabbit skin during transdermal iontophoresis. *J Pharm Sci* **92**: 1482-8 (2003).
3. M. B. Delgado-Charro and R. H. Guy. Characterization of convective solvent flow during iontophoresis. *Pharm Res* **11**: 929-35 (1994).