M1165

In Vitro Optimization of Amikacin Reverse Iontophoresis

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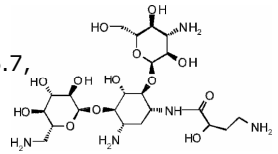
MW: 585.6
pKa: 8.4, 6.7, 9.7, 8.4



AIM OF THE WORK

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.

We intended to optimize AK reverse iontophoresis extraction across the skin. Objectives of the work were to examine the effect of the composition of the extracting buffer, replacing saline solution with citrate buffer, on AK extraction. Acetaminophen (AM), a polar non-ionized molecule, was used as marker, to quantify electroosmotic contribution to overall AK extraction.

METHODOLOGY

Reverse iontophoresis:

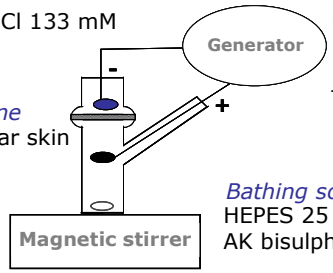
I = 0.5 mA/cm²

Anodal iontophoresis
HEPES 25 mM; NaCl 133 mM
- pH 4.0
- pH 8.0

Cathodal iontophoresis
HEPES 25 mM; NaCl 133 mM
- pH 4.0
- pH 8.0

Trisodium citrate 25 mM or 50 mM pH 8

Membrane
Rabbit ear skin



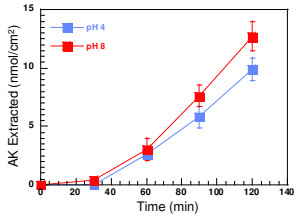
Bathing solution
HEPES 25 mM; NaCl 133 mM; pH 7.4
AK bisulphate 200 µM; AM 1 mM.

AK HPLC analysis:

Samples derivatizated with 1-fluoro-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

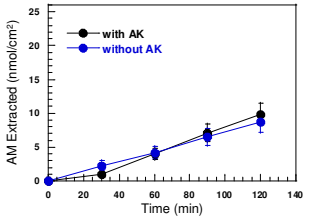
AK cathodal extraction at different pH (average ± sd)



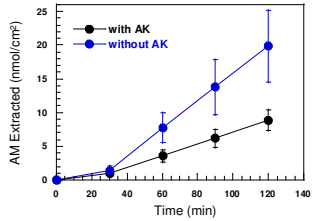
The extraction of AK was independent of pH and always in the anode-to-cathode direction, in agreement with the positive charge of the drug.

AM cathodal extraction at different pH (average ± sd)

pH 4

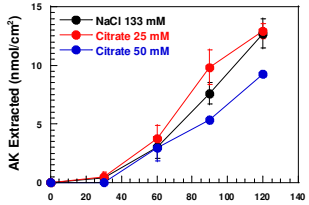


pH 8



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 of the skin, reducing electroosmotic flow.

AK cathodal extraction with different buffers (average ± sd)



AK extraction is affected by the ionic strength (µ) of the buffer used as extraction solution:
- **NaCl 133 mM µ= 133 mM**
- **Citrate 25 mM µ= 187.5 mM**
- **Citrate 50 mM µ= 300 mM**

AM cathodal extraction with different buffers (average ± sd)

Extraction Buffer	AM extracted (nMol/cm²)		AK extracted (nMol/cm²)
	Without AK	With AK	
NaCl 133 mM	19.86±4.27	8.79±1.52	12.73±3.09
Citrate buffer 25 mM	19.55±0.62	18.91±5.85	12.90±1.11
Citrate buffer 50 mM	20.64±3.25	14.68±2.01	9.29±0.33

The increase of ionic strength does not influence AM extraction in this experimental conditions. The amount of AM extracted using a citrate buffer is the same with or without AK in the bathing solution, probably because the use of a citrate buffer neutralizes the positive charges of AK interacting with the skin.

CONCLUSIONS

- ✓ ***AK extraction takes place at the cathode at both pH 4.0 and 8.0***
- ✓ ***The main mechanism involved in AK extraction is electromigration***
- ✓ ***Positively charged AK alters skin permeselectivity, interacting with negatively charged skin and reducing electroosmotic flow***
- ✓ ***The main factor affecting AK cathodal extraction resulted the ionic strenght of the solution***
- ✓ ***The use of a citrate buffer does not modify electroosmotic flow, but neutralize the positive charges of AK buond to the skin***

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).