The aim of this study was to determine aztreonam (ATM) membrane permeability using Calu-3 cells and its plasma and pulmonary epithelial lining fluid (ELF) pharmacokinetics in rats after intratracheal nebulization and intravenous administration (15 mg · kg⁻¹). ATM exhibits low Calu-3 permeability (0.07 ± 0.02 × 10⁻⁶ cm · s⁻¹), and a high area under the ELF/unbound plasma concentration time curve between 0 and infinity (AUC_{ELF}/AUC_{unplasma}) ratio of 1.069 was observed after nebulization in rats. These results confirm that ATM is a low-permeability molecule and a good candidate for nebulization.

Antibiotic inhalation is frequently combined with systemic administration to treat lung infections (1). The rationale for inhaled antibiotics is to obtain high concentrations at the target site (i.e., lungs) and low systemic concentrations and therefore limited side effects (2). Three antibiotics are currently available for pulmonary administration: tobramycin (TOB), colistin (CST) administered as colistin methansulfonate (CMS), and aztreonam (ATM) (2). We have previously shown in healthy rats that with TOB and CST, much higher concentrations were obtained within the lungs after nebulization (NEB) than after intravenous (i.v.) administration (3, 4). The aim of this study was to confirm these results with ATM, using the same standardized protocol.

ATM for parenteral administration was used for all experiments (1 g Azactam; Sanofi Aventis). The membrane apparent permeability (P_{app}) was measured by in vitro transepithelial transport experiments across Calu-3 monolayers (5, 6). Briefly, cells were cultured on Transwell inserts (Corning) for 15 days, and on the day of the experiment, they were incubated in the presence of 250 μg · ml⁻¹ ATM in the donor compartment. After 60 min of incubation, the ATM concentration was measured in the acceptor compartment. The P_{app} measurements were obtained from two different experiments and in triplicate (n = 6). The animal experiments were approved by the local ethics committee (COMETHEA) and registered by the French Ministry of Higher Education and Research (no. 01733.01). Briefly ATM was administered under anesthesia at a dose close to 15 mg · kg⁻¹, either by an i.v. bolus in the tail vein (1 ml) or by intratracheal nebulization (NEB) (100 μl, MicroSprayer model 1A-1B; Penn-Century, Wyndmoor, PA, USA) (7) in two groups of rats (male Sprague-Dawley rats; n = 27, 307 ± 15 g for the i.v. group and n = 26, 302 ± 15 g for the NEB group; Janvier Laboratories, Le Genest-Saint-Isle, France). Bronchoalveolar lavage (BAL) fluid and blood samples were collected at 0.25, 0.5, 1, 2.5, and 4 h after administration (4 to 7 rats per sampling time) in both groups. Assays were conducted by liquid chromatography-tandem mass spectroscopy (LC-MS/MS). The system included an Agilent high-performance liquid chromatography (HPLC) system module (HP1100; Agilent Technologies, Les Ulis, France) coupled with an API 3000 mass spectrometer (Sciex, Les Ulis, France). An XBridge C_{18} column (5.0 μm, 150 by 2.1 mm inside diameter [ID]; Waters, Saint-Quentin en Yvelines, France) was used, and a mobile phase composed of 2 mM ammonium acetate and acetonitrile (75:25 [vol/vol]) was delivered isocratically at 0.2 ml · min⁻¹. The mass spectrometer was operated in the positive mode. Ions were analyzed by multiple reaction monitoring (MRM). The transitions were m/z 436.1/313 for ATM and 442.1/313 for its deuterated

<table>
<thead>
<tr>
<th>Drug</th>
<th>Molar mass (g · mol⁻¹)</th>
<th>Log P*</th>
<th>P_{app} (10⁻⁶ cm · s⁻¹)</th>
<th>AUC_{ELF}/AUC_{unplasma} ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>435.4</td>
<td>−3.1</td>
<td>0.07 ± 0.02</td>
<td>1,069</td>
</tr>
<tr>
<td>TOB</td>
<td>467.5</td>
<td>−6.5</td>
<td>&lt;0.05</td>
<td>230</td>
</tr>
<tr>
<td>CST</td>
<td>1166</td>
<td>−8.1</td>
<td>0.04 ± 0.02</td>
<td>1,214</td>
</tr>
</tbody>
</table>

* Data obtained from Chemaxon Software (www.drugbank.ca).

* Data from Marchand et al. (3).

* Data from Gontijo et al. (4).
The ATMPapp, in Calu-3 monolayers was estimated to be 0.02 ± 0.02 × 10−6 cm · s−1, which is relatively low but close to the values found for CST and TOB (Table 1) (3, 4) and lower than those estimated under similar experimental conditions for ciprofloxacin (CIP) and moxifloxacin (MOX) (10). The ATMP total plasma maximum concentrations derived from the model were significantly lower after NEB than after i.v. administration (16.2 ± 5.5 μg · ml−1 versus 34.3 ± 6.6 μg · ml−1 at t = 0.5 h) (P < 0.05; Mann-Whitney test), and the t1/2plasma was almost 3 times higher after NEB than after the i.v. bolus (t1/2plasma,NEB = 0.98 h versus t1/2plasma,i.v. = 0.34 h), suggesting that ATMP absorption from the lung is the elimination rate-limiting step (Fig. 1). The ratio of the plasma AUCs after NEB and i.v. administrations was 1.09, indicating that with the Penn-Century system the dose administered was fully delivered and then absorbed systemically. The ATMP ELF concentrations were much higher after NEB than after the i.v. bolus at the same dose (Fig. 1), with an AUCELF/AUCu,plasma ratio that was approximately 2,673 times higher (1,069 versus 0.39) (Table 1). The AUCELF/AUCu,plasma ratio being lower than unity after the i.v. bolus (Table 1) suggests that ATMP could be the substrate of a transporter, limiting its penetration within ELF after systemic administration, although to our knowledge, no such transporters have been described, but it could also be that ATMP slowly distributes into the ELF. This low AUCELF/AUCu,plasma ratio after i.v. administration also differentiates ATMP from TOB and CST (Table 1). However, this ratio is noticeably close to 1,000 for ATMP after NEB, consistent with the value obtained with CST (1,214) (4) and several fold higher than that obtained with TOB (230) (3), for reasons that still need to be explained (Table 1). Yet apart from these differences, ATMP, CST, and TOB essentially present similarities. They are hydrophilic (low log P) with low permeability (low Papp), leading to mostly similar biopharmaceutical characteristics after nebulization (Table 1). Therefore, together with the two previous studies conducted under similar experimental conditions (3, 4), this new investigation provides a biopharmaceutical rationale for using ATMP, CST, and TOB as aerosols for the treatment of pulmonary infections, consistent with the current practice. Complementary experiments are now being conducted with other compounds presenting different characteristics in terms of solubility and permeability that will constitute the basis of a biopharmaceutical classification of nebulized antimicrobial agents.

ACKNOWLEDGMENT

We thank Hélène Fayard for her technical assistance in this study.

REFERENCES

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