Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 5. Oseltamivir Carboxylate

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The aim of this study was to determine the biopharmaceutical characteristics of oseltamivir carboxylate (OC) after pulmonary delivery. After OC bolus and intratracheal nebulization (NEB) in rats, blood was collected and bronchoalveolar lavages (BALs) were performed. Epithelial lining fluid (ELF) concentrations were estimated from BAL fluid. The area under the curve (AUC) ratio for ELF to plasma was 842 times higher after NEB than after intravenous (i.v.) administration, indicating that OC nebulization offers a biopharmaceutical advantage over i.v. administration.

Inhalation of antimicrobial agents may benefit the treatment of lung infections by providing high concentrations at the site of infection and low systemic concentrations. However, this potential advantage may vary with the type of nebulizer as well as with drug characteristics such as, in particular, permeability. We have recently developed a standardized experimental setting to compare the biopharmaceutical characteristics of nebulized antimicrobial agents in healthy rats, eventually leading to a biopharmaceutical classification of inhaled antimicrobial agents. The initial experiments have demonstrated major differences between antibiotics with high membrane permeation ability, such as fluoroquinolones (1), and those with much lower membrane permeation ability, including colistin (2), tobramycin (3), and aztreonam (4), which are actually currently aerosolized in patients. But, because pulmonary infections may also be due to viruses, the objective of this new study was to compare the intrapulmonary pharmacokinetics (PK) of an anti-influenza drug after systemic administration and nebulization (NEB) in order to assess the potential benefit of direct pulmonary delivery. Oseltamivir is a potent and selective inhibitor of neuraminidase. It was selected for this study because it is the most widely used antiviral drug for the treatment and prophylaxis of influenza A and B virus infections (5). However, in order to improve its bioavailability, it is administered orally in clinical practice as an ethyl ester prodrug, oseltamivir phosphate (OP), which is then rapidly hydrolyzed into the active oseltamivir carboxylate (OC) (6). Yet, because the objective of nebulization is to maintain high presystemic concentrations of the active moiety, direct nebulization of OC instead of OP should be a better alternative. The aim of this study was to investigate the biopharmaceutical characteristics of nebulized OC in healthy rats using our standardized protocol (1–4), first, in order to assess the potential interest of this alternative route of administration in clinics, and second, to extend the number of compounds that will be necessary for setting a biopharmaceutical classification of inhaled antimicrobial agents.

The animal experiments were conducted in compliance with EC Directive 2010/63/EU after approval by the local ethics committee (COMETHEA) and were registered by the French Ministry of Higher Education and Research under authorization number 2015070211159865. The complete experimental setting was pre-

viously reported (1, 2). Briefly, OC (oseltamivir acid; Alsachim, Illkirch Graffenstaden, France) was administered under anesthesia at a dose close to 6 mg · kg of body weight⁻¹ either by intravenous (i.v.) bolus into the tail vein (1 ml) or by intratracheal nebulization (NEB; 100 µl; Microsprayer 1A-1B; Penn-Century, Wyndmoor, PA, USA) in two groups of male Sprague-Dawley rats ($n = 17$, 324 ± 23 g, for i.v. group and $n = 15$, 322 ± 20 g, for NEB group; Janvier Laboratories, Le Genest-St-Isle, France). In both groups, bronchoalveolar lavage (BAL) fluid and blood samples were collected 0.5, 2, 4, and 6 h after OC administration (3 to 5 rats per sampling time) and extra samples were collected at 1 h after i.v. administration. OC was assayed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a previously described method with minor adaptation (7). A Shimadzu Nexera X2 separation module coupled with an ABSciex API 3000 tandem mass spectrometer was used. Chromatographic separation was carried out on an X Bridge BEH300 C₁₈ 300A column (5.0 µm, 150 by 2.1-mm inside diameter [i.d.]; Waters, St-Quentin en Yvelines, France). The mobile phase was composed of acetonitrile-ammonium formate (10 mM)-water (50:10:40, vol/vol), and the flow rate was 0.2 ml · min⁻¹. The mass spectrometer was operated in the positive ion mode. Ions were analyzed by multiple-reaction monitoring (MRM). Transition ions were m/z 285.1 > 138.0 for OC and 289.2 > 138.0 (¹³C, ²H₃ OC) for deuterated internal standard. Ten-point calibration standards with concentrations between 0.625 and 375 ng · ml⁻¹ and 3 levels of control were prepared. The intraday and interday variability in plasma and BAL fluid was determined at three levels of concentrations with a pre-

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FIG 1 Predicted concentration-time profiles of oseltamivir carboxylate ($6 \text{ mg} \cdot \text{kg}^{-1}$) in plasma (solid line) and in ELF (dashed line) from simultaneous PK modeling after i.v. and NEB administrations. Closed and open symbols represent means $\pm$ standard deviations of experimental concentrations in plasma (total) and in ELF, respectively.

cision and accuracy of less than 15%. The limit of quantification (LOQ) in both media was set at $0.625 \text{ ng} \cdot \text{mL}^{-1}$. Concentrations of urea in plasma and BAL fluid were measured as previously described (2, 8). OC concentrations in epithelial lining fluid (C_{ELF}) were derived from measured BAL fluid concentrations (C_{BAL}) after correction by urea dilution (8). OC concentrations versus time in plasma and ELF were simultaneously analyzed by a nonlinear mixed-effect method with S-ADAPT software (v.1.52). Two compartments were used to describe OC pharmacokinetics (PK) in plasma. OC PK in ELF was tested with one and two compartments, and two compartments with distinct volumes (V_{ELF1} and V_{ELF2}) were kept in the final model as previously described with tobramycin (3). Compartments were connected by two-direction equilibrium distribution clearances and by the addition of an efflux clearance from the central compartment to the ELF1 compartment (CL_{out}). Only unbound drug in plasma was assumed to penetrate within ELF (4), but OC protein binding was considered to be negligible (6). Areas under plasma concentrations and ELF concentration-versus-time curves from 0 to infinity ($\text{AUC}_{\text{plasma}}$ and AUC_{ELF} , respectively) were calculated from the model. Elimination half-lives ($t_{1/2,\text{plasma}}$ and $t_{1/2,\text{ELF}}$) after i.v. administration and NEB were derived from the model (Berkeley Madonna, version 8.3.18; University of California).

This study was able to demonstrate a major effect of the route of administration on OC intrapulmonary PK, with much higher ELF concentrations after nebulization than after i.v. administration, and virtually identical plasma concentrations independent of the route of administration, as illustrated in Fig. 1. Lung exposure may be characterized by AUC_{ELF} estimated by the model, which was 550-fold higher after nebulization than after i.v. administration (Table 1). This effect of the route of administration may be further evidenced by the much higher ELF/plasma AUC ratio after

nebulization ($\text{AUC}_{\text{ELF,NEB}}/\text{AUC}_{\text{plasma,NEB}} = 842$) than after i.v. administration ($\text{AUC}_{\text{ELF,i.v.}}/\text{AUC}_{\text{plasma,i.v.}} = 1.01$) (Table 1). Another interesting observation was that whatever the route of administration, OC concentrations peaked early, more precisely at 0.5 h, corresponding to the first sampling time, both in plasma and in ELF (Fig. 1). Elimination half-life values in plasma and in ELF are presented in Table 1.

The important effect of the route of administration on OC pulmonary PK has already been observed, with antibiotics such as colistin (2), tobramycin (3), and aztreonam (4) presenting low apparent permeability (P_{app}) across Calu-3 cell monolayers (9), but not with fluoroquinolones, which are characterized by higher P_{app} (10). Therefore, OC presents the characteristics of low membrane permeability, consistent with its poor oral availability, which explains why it is not administered directly (11), and with its relatively low log D value at pH 7.4 (-2.1) (12). Unfortunately, OC P_{app} across Calu-3 cells could not be properly estimated because of analytical limitations as previously encountered with tobramycin due to low membrane permeability (3). This should be reassessed, and complementary experiments should also be conducted after nebulization with OP in order to better appreciate the benefit of nebulizing OC directly instead of OP.

Although these results cannot be directly extrapolated to the clinical setting, they clearly demonstrate that much higher OC ELF than plasma concentrations may be obtained by direct nebulization of this active moiety, which may present a potentially major interest for the treatment of pulmonary infections due to influenza A and B viruses.

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TABLE 1 Areas under plasma concentrations and ELF concentrations versus time curves from 0 to infinity ($\text{AUC}_{\text{plasma}}$ and AUC_{ELF}) and half-lives ($t_{1/2}$) estimated by the model

Parameter	i.v.	NEB
$\text{AUC}_{\text{inf,plasma}} (\mu\text{g} \cdot \text{h} \cdot \text{mL}^{-1})$	6.21	4.11
$\text{AUC}_{\text{inf,ELF}} (\mu\text{g} \cdot \text{h} \cdot \text{mL}^{-1})$	6.29	3,463
$\text{AUC}_{\text{inf,ELF}}/\text{AUC}_{\text{inf,plasma}}$	1.01	842
$t_{1/2,\text{plasma}}$	1.8	1.9
$t_{1/2,\text{ELF}}$	2.3	1.4

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