Population Pharmacokinetic Analysis of Colistin in Burn Patients

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Colistin is increasingly used as a salvage therapy for nosocomial infections caused by multidrug-resistant Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. However, the available pharmacokinetic (PK) data for colistin are limited to guide dosing. The aim of this study was to develop a population PK model of colistin and to identify the optimal dosage regimens for burn patients. Fifty patients with burns ranging from 4% to 85% of total body surface area who had been treated with colistimethate sodium (CMS) were studied. CMS, which is hydrolyzed in vivo to an active metabolite, was intravenously administered every 12 h. Blood samples were collected at 0, 1, 2, 4, 6, and 8 h after more than five infusions to measure the colistin concentration using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. The population PK model was developed using nonlinear mixed effect modeling (NONMEM, v. 6.2). A one-compartment linear PK model for colistin best described the data. The covariates included in the final model were creatinine clearance for the relative fraction of bioavailability and continuous renal replacement therapy (CRRT). The aim of this study was to develop a population PK model for colistin after intravenous administration of colistimethate sodium (CMS; SteriMax Inc., Mississauga, Ontario, Canada) in burn patients.

**MATERIALS AND METHODS**

**Patients.** Fifty patients with burns ranging from 4% to 85% of total body surface area (TBSA) were treated with CMS. They were admitted to the Burn Intensive Care Unit (BICU) of Hangang Sacred Heart Hospital between June 2010 and May 2011. The study protocol was approved by the Institutional Review Board of Hangang Sacred Heart Hospital, and all procedures, including written informed consent by the patients or legal representatives (in cases in which the patient could not give consent) were conducted in accordance with the principles of the Declaration of Helsinki and the Korean Good Clinical Practice guidelines. Patients who were pregnant, breastfeeding, <18 years old, or allergic to CMS/colistin were excluded. The demographic characteristics are summarized in Table 1. For each patient, the following data were recorded on the first day of colistin administration: age, sex, body weight, TBSA, abbreviated burn severity index (ABSI), acute physiology and chronic health evaluation II (APACHE II) score, serum creatinine, serum albumin, and the presence of edema (referred to here simply as edema), sepsis, dehydration, and continuous renal replacement therapy (CRRT).

**CMS administration and blood sampling.** CMS (150 mg as colistin base activity [CBA]) was intravenously administered every 12 h over 30 min; 150 mg CBA dissolved in 100 ml of normal saline is equivalent to 5 million units. Venous blood samples (5 ml) for the measurement of plasma colistin concentrations were collected in heparinized tubes from...
TABLE 1 Demographic data for the enrolled patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. or mean (SD)</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Sex (male/female)</td>
<td>39/11</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>48 (13)</td>
<td>26–80</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>65.8 (10.3)</td>
<td>50–98</td>
</tr>
<tr>
<td>TBSA affected (%)</td>
<td>50.5 (21.8)</td>
<td>4–85</td>
</tr>
<tr>
<td>ABSI</td>
<td>9.82 (2.34)</td>
<td>5–14</td>
</tr>
<tr>
<td>Days after injurya</td>
<td>15.5 (10.4)</td>
<td>3–58</td>
</tr>
<tr>
<td>CLCR (ml/min)*</td>
<td>128 (75.2)</td>
<td>22.6–309</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.5 (0.3)</td>
<td>1.9–3.1</td>
</tr>
<tr>
<td>Sepsis (yes/no)</td>
<td>29/21</td>
<td></td>
</tr>
<tr>
<td>Edema (yes/no)b</td>
<td>18/32</td>
<td></td>
</tr>
<tr>
<td>CRRT (yes/no)</td>
<td>17/33</td>
<td></td>
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</table>

a The range was 11 to 85% for all but one patient, who had an electrical burn over 4% of the body surface area. Types of injury were as follows: flame burn (n = 39), electrical burn: (n = 5), scalding burn (n = 3), chemical burn (n = 2), and contact burn (n = 1).

b Days after injury, days from burn injury to the initiation of CMS treatment.

c CLCR estimated by the Cockcroft-Gault equation.

d Clinical diagnosis (puffy face and pitting edema in the legs).

The in-dwelling catheter of the central or peripheral vein at 0 (before dosing), 1, 2, 4, 6, and 8 hours after the end of the infusion of colistin. PK sampling was performed at least 3 days after the first dose of CMS so that colistin plasma concentrations could reach the steady state. The actual times of CMS administration and blood sampling were recorded. Samples were kept in an ice-cold bucket until centrifugation at 2,092 × g for 10 min at 4°C. The centrifugation was done within 0.5 h after sampling. Separated plasma samples were stored at −70°C to prevent in vitro conversion of CMS to colistin (17).

**Determination of colistin concentrations in plasma.** Colistin A and colistin B concentrations in plasma were determined by high-performance liquid chromatography (1C; Agilent 1200 series chromatograph; Agilent Technologies, Santa Clara, CA) coupled with a tandem mass spectrometer (MS/MS; AB SCIEX API 3200 LC-MS/MS system; Applied Biosystems, Foster City, CA) method (18, 19). In brief, the assay method was as follows. A volume of 100 μl of plasma was precipitated with 400 μl acetonitrile containing an internal standard (polymyxin B, 5 μg/ml). After thorough vortexing for 1 min, the samples were centrifuged at 13,523 × g for 10 min at 4°C. A volume of 200 μl of the supernatant was diluted with 200 μl of 0.1% formic acid, and 5 μl was injected into an LC-MS/MS system. The analytes were separated through an Xbridge C18 column (100 mm by 2.1 mm, 5 μm) at a flow rate of 300 μl/min. The mobile phases were 0.1% formic acid in distilled water (vol/vol) (mobile phase A) and 0.1% formic acid in acetonitrile (vol/vol) (mobile phase B). The separation was achieved using the gradient program. The detection was performed in the electrospray negative-ion mode of tandem mass spectrometry. Mass-to-charge ratios (m/z) in multiple reaction monitoring were 585.5 to 101.2 for colistin A, 578.5 to 101.2 for colistin B, and 602.5 to 241.2 for polymyxin B.

The lower limits of quantification were 70.35 ng/ml and 134.7 ng/ml for colistin A and colistin B, respectively. The coefficients of correlation were greater than 0.9966 in the range of about 70.35 to 7,035 ng/ml for colistin A and 0.9963 in the range of 134.7 to 13,470 ng/ml for B by weighted linear regression (1/concentration). Intra- and interday precision (relative standard deviation) and mean accuracy ranging from 211.1 ng/ml to 5,628 ng/ml were below 8.62% and 96.63% to 108.3%, respectively. The samples for quality control were prepared separately from those for the calibration curve.

**Population PK model development.** A nonlinear mixed-effects model analysis was conducted using NONMEM version 6.2 (Icon Development Solutions, Ellicott City, MD) with the G77 FORTRAN compiler. The first-order conditional estimation method with interaction was used throughout the model building process.

Because the plasma concentration-time curves of colistin A and colistin B were almost identical, the sums of the plasma concentrations of colistin A and colistin B were used for PK modeling (9). Since the exact fraction of CMS converted to colistin cannot be determined without concentration profiles after direct infusion of colistin and the active metabolite, we had to employ another scaling parameter, fm*, instead of the fraction. fm* was defined as the theoretical fraction of CMS that is expected to be converted to colistin when renal excretion of CMS does not happen at all (i.e., CLCR = 0 ml/min). The concept of relative fraction of CMS converted to colistin (RFM) was also used to reflect the influence of renal function on the fraction: RFM = 1 − θ2 × (CLCR/128), where θ2 is the slope of fm* decreased by CLCR (ml/min) centered on its median value (128 ml/min) in our patients. These two parameters, fm* and RFM, were used to define the fraction of CMS converted to colistin, fm: fm* = fm × RFM, where fm is the unknown fraction of CMS converted to colistin. RFM is not measurable, but it is estimable from the comparison of fm* between patients. The apparent PK parameters of CL/fm and V/fm were re-estimated as CL/fm*, V/fm*, and RFM (fm* = fm × RFM) here. The fm* or RFM was estimated as the denominator of the apparent CL or V, not as a separate parameter, throughout these steps.

As for V, one- and two-compartment distribution models with first-order elimination were tested for the plasma concentration-time profile of colistin. However, CMS was assumed to distribute in a single compartment only, as CMS concentrations were not measured in our study. TR, the first-order rate constant for turnover of CMS to colistin, was also put into the model (Fig. 1). The subroutine chosen to build the model was ADVAN2 TRANS2 in the NONMEM library, which was parameterized in terms of CL, V, F1, and K1.

The interindividual variability of each parameter was applied exponentially: Pi = θi × exp(ηi), where Pi is the i th parameter of the ith individual, θi is the typical value of the jth population parameter, and ηi is a random variable, normally distributed with a mean of 0 and variance of σi2. The residual error models evaluated were additive, proportional, and combined.

Models were selected based on several criteria, such as diagnostic scatter plots, an objective function value (OFV) decrease of 3.84 (α = 0.05, df = 1) for nested models, and the Akaike information criterion for non-nested models. The residual-based model diagnostic was performed using conditional weighted residuals.

The covariate model building was performed in a stepwise fashion with forward inclusion and backward deletion. The variables suspected to be significant covariates were age, sex, body weight, creatinine clearance (CLCR), TBSA, ABSI, APACHE II score, serum creatinine, serum albumin, days from burn injury to the initiation of colistin therapy, edema, and the presence of sepsis, dehydration, and CRRT. CLCR was estimated from the Cockcroft-Gault equation (20) using the age, body weight, and serum creatinine level of each subject.

FIG 1 Pharmacokinetic structural model for colistin. TR, turnover rate constant of CMS converted to colistin; RFM, relative fraction of CMS converted to colistin.
The covariate screening process was performed using visual (parameter-versus-variable scatter plots) and numerical (generalized additive modeling implemented in Xpose [v. 4]) approaches. Variables that passed the screening procedures were included in the model and tested for significance as a covariate based on aforementioned model selection criteria. At the backward elimination step, covariates that did not increase the minimized OFV more than 6.63 ($\alpha = 0.01, df = 1$) were eliminated from the final model.

**Bootstrapping and visual predictive checks (VPCs).** The bootstrap resampling method was used to evaluate the stability and robustness of the final PK model. Resampling with replacement generated 1,000 bootstrap data sets, and the final population PK model was fitted repeatedly to each of them. Ninety-five percent confidence intervals (CIs) for the final parameters were obtained from the bootstrap empirical posterior distribution.

VPCs were performed by overlaying observed data points with 5th, 50th, and 95th percentile curves of 1,000 data sets simulated from the final model.

**Simulation of colistin AUC.** The steady-state values for the area under the concentration-time curve from 0 to 24 h (AUC$_{0–24}$) for colistin on the basis of the model developed for the currently used dosage regimen (150 mg as a 30-min infusion every 12 h) were obtained from 1,000 simulated virtual burn patients. In addition, we simulated the steady-state colistin AUC$_{0–24}$ values for different levels of renal function according to the total daily dose of CBA.

**RESULTS**

**Final PK model.** A one-compartment linear PK model with combined residual errors for colistin best described the data (Fig. 1). The parameters of the basic model were CL$/f_{m}^{\star}$, V$/f_{m}^{\star}$, RFM, and TR. Covariates included in the final model were CL$_{CR}$ for the RFM and the presence of edema for TR. The incorporation of CL$_{CR}$ and the presence of edema decreased the OFV by 15.1 and 10.0, respectively.

The estimated parameter of RFM ($\theta_4$) was 0.213, which shows the change in the fraction of CMS converted to colistin by renal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>% RSE</th>
<th>Bootstrap median (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>Structural model</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CL$_{col}$ = $\theta_1$/RFM</td>
<td>8.49 liters/h</td>
<td>7.04</td>
<td>8.52 (6.94–10.9)</td>
</tr>
<tr>
<td>$\theta_1$ (CL$/fm^{\star}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V$_{col}$ = $\theta_2$/RFM</td>
<td>81.1 liters</td>
<td>9.74</td>
<td>81.4 (59.6–113.5)</td>
</tr>
<tr>
<td>$\theta_2$ (V$/fm^{\star}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR = $\theta_3$ – edema $\times \theta_5$</td>
<td>0.796 h$^{-1}$</td>
<td>16.8</td>
<td>0.798 (0.578–1.19)</td>
</tr>
<tr>
<td>$\theta_3$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\theta_5$</td>
<td>0.425 h$^{-1}$</td>
<td>31.5</td>
<td>0.429 (0.174–0.721)</td>
</tr>
<tr>
<td>RFM = 1 − $\theta_4$ × (CL$_{CR}$/128)</td>
<td>0.213</td>
<td>18.6</td>
<td>0.211 (0.151–0.268)</td>
</tr>
</tbody>
</table>

| Interindividual variability | | | |
| $\omega_{CL_{col}}$ | 37.5% | 9.47 | 36.8 (29.8–41.2) |
| $\omega_{V_{col}}$ | 24.3% | 26.9 | 23.2 (1.48–37.4) |
| $\omega_{TR}$ | 67.2% | 16.9 | 65.6 (40.4–88.2) |

| Residual error | | | |
| $\sigma_{add}$ | 99.2 ng/ml | 23.7 | 96.3 (0.13–139) |
| $\sigma_{prop}$ | 6.72% | 30.8 | 6.63 (2.27–11.3) |

*CL$_{col}$, apparent clearance of the central compartment for colistin ($\theta_1$, the minimum value of CL$/fm^{\star}$); V$_{col}$, apparent volume of distribution of the central compartment for colistin ($\theta_2$, minimum value of V$/fm^{\star}$); TR, turnover rate constant of CMS converted to colistin ($\theta_3$, TR for nonedematous patients; $\theta_5$, TR difference based on the presence of edema); RFM, relative fraction of CMS converted into colistin ($\theta_4$, the slope of RFM change by CL$_{CR}$); RSE, relative standard error; $\sigma_{add}$, additive error; $\sigma_{prop}$, proportional error.

b 95% CI estimated by applying the final population PK model to 1,000 resampled datasets.
function. This is interpreted to mean that an anephric patient (CLCR = 0 ml/min) converts CMS to colistin at about 5 times the fraction in a patient with normal renal function (CLCR = 128 ml/min), although the absolute fraction was not measured.

In Table 2, estimated apparent CL and V are presented as CL/($f_m^* \times \text{RFM}$) and V/($f_m^* \times \text{RFM}$), where the RFM ranges between 0 and 1. When the RFM is 1 in an anephric (CLCR = 0 ml/min) patient, his/her apparent CL and V will be their minimum values (CL/$f_m^*$ and V/$f_m^*$). The minimum was estimated as $\theta_1$ (8.49 liters/h) and $\theta_2$ (81.1 liters) in the model. For a patient with a CLCR of 128 ml/min, the RFM equals 0.787 ($1 - 0.213 \times \text{CLCR}/128$), and the typical values of apparent CL and V were expected to be 10.8 liters/h (8.49/0.787 liters/h) and 103 liters (81.1/0.787 liters), respectively.

The basic goodness-of-fit plots for the final PK model are presented in Fig. 2 and demonstrate that individual predicted colistin concentrations corresponded well to observations without systemic bias. The median parameter estimates and 95% confidence intervals from 1,000 bootstrap replications are summarized in Table 2. The median parameter estimates obtained from the bootstrap method were very similar to the estimates of the final PK model. VPCs of the final population PK model are shown in Fig. 3 and stratified by the edema status (with or without edema) and CLCR values (<70 ml/min or ≥70 ml/min). The model-predicted confidence intervals as well as medians corresponded adequately to the observed data.

Simulation of colistin AUC. The frequency distribution of AUC$_{0-24}$ for 1,000 virtual burn patients treated with the dosing regimen in this study was predicted based on the final PK parameter estimates (mean ± SD, 29.2 ± 13.5 µg/ml·h) (Fig. 4). The changes in mean predicted AUC$_{0-24}$ for different levels of renal function (CLCR < 70 ml/min and CLCR ≥ 70 ml/min) relative to

FIG 3 Visual predictive check plots of the final population PK model classified by edema (top) and CLCR (bottom). Circles, observations; solid black lines, median simulated curve; dashed black lines, 5% and 95% prediction intervals; solid blue lines, median observed curve; dashed blue lines, 5th and 95th percentiles of observations.

FIG 4 Histogram of 1,000 simulated AUC$_{0-24}$ values with the final pharmacokinetic parameter estimates after dosing with 150 mg of colistin base activity twice daily.
colistin daily doses (CBA from 300 mg to 500 mg) are shown in Fig. 5.

DISCUSSION

The terminal elimination half-life (t_{1/2}) of colistin estimated herein was 6.6 h. It was much shorter than that in critically ill patients (t_{1/2} = 14.4 h) reported by Plachouras et al. (14). This discrepancy seems to be due to the difference in the apparent V of colistin, which was double the value estimated in our burn patients, while the apparent CL of colistin was similar.

Both the t_{1/2} (4.98 h) and apparent V (67.9 liters) obtained using noncompartmental analysis in a previous study in healthy volunteers (21) were lower than those estimated in this study, but the apparent CL was similar. In general, increased blood flow to the kidneys and liver in the hypermetabolic phase (beyond 48 h after the burn injury) (16) and massive hydration in the treatment process increase the CL and V of many antibiotics. However, the elimination mechanism of colistin is not known, and the fact that the CL in our report is similar to values in unburned critically ill patients suggests that it is not affected by the hypermetabolism in burn patients.

Two recent studies with ICU patients who received 270 mg of CBA daily showed the incidences of nephrotoxicity to be 18.6% and 14.3%, respectively (7, 22). Serum creatinine levels were more than doubled in 4 patients during colistin therapy in this study, although the causal relationship was not clear. Colistin was discontinued for a patient and halved for another among the 4 patients with suspected toxicity. Because the frequency of toxicity caused by daily doses higher than the approved regimen is rarely reported, physicians should carefully assess the relationship between safety and CL_{CR} if they consider dose escalation in burn patients, as exemplified in Fig. 5, where the AUCs at several different doses were predicted assuming PK linearity.

Since the plasma concentrations of CMS were not measured in this study, PK parameter estimates for CMS are not given. Instead, RFM and TR were used to build the PK model for colistin, the active metabolite. In addition, the meaningful covariates (CL_{CR} ~ RFM, edema ~ TR) might be a physiological explanation for the changes in CL and V for CMS.

Renal function, expressed as CL_{CR} was not a significant covariate affecting CL for colistin, which was consistent with previous results indicating that colistin is predominantly eliminated by the nonrenal route (23, 24). In the final PK model, CL_{CR} was the only significant covariate for RFM as given in the final model: RFM = 1 − θ_{4} × (CL_{CR}/128). The inversely proportional relationship between RFM and CL_{CR} in our model was in agreement with the report that CMS is eliminated mainly via the kidney (23, 24). Patients with normal glomerular function (typical CL_{CR} = 128 ml/min) appeared to have an RFM (0.797) lower than that (RFM = 0.967) in patients with renal failure (typical CL_{CR} = 20 ml/min), because the amount of CMS, the precursor of colistin, in systemic circulation is smaller. When the plasma concentrations of CMS were measured in the present study, CL_{CR} might have been a significant covariate for CL of CMS, similar to the results of Garonzik et al. (12).

TR, the turnover rate constant of CMS converted to colistin, was significantly smaller in burn patients with edema (0.371 h^{-1} for edematous patients versus 0.796 h^{-1} for nonedematous patients). However, there is no reported evidence or suggestion that the constant of the rate of hydrolysis of CMS to colistin is attenuated by edema. Rather, it seems to be related to the interstitial fluid retention causing an increased V of CMS in edematous patients (25). The increased V causes lower serum concentrations of CMS and, in turn, the lower turnover rate, which was defined as TR × CMS concentration. Because no PK parameters or concentrations of CMS were modeled in our study, we infer that lower turnover rates in edematous patients cannot but appear as the lower estimated TR in our PK model.

Although the study by Garonzik et al. (12) showed that CRRT affected the disposition of CMS and colistin, CRRT was not acknowledged as a significant covariate for any PK parameters in this study. In addition, individual CL and RFM in patients with and without CRRT did not show significant difference in t tests (results not shown).

This is the first population PK analysis of colistin in burn patients. Unlike in previous studies with critically ill patients, we found that the half-life of colistin was much shorter and that only CL_{CR}, not CRRT, was the significant covariate for the CL of colistin. The population PK model developed here may be applied for the optimization of colistin dosage regimens in burn patients in the future.

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REFERENCES


