Reduced subcutaneous tissue distribution of cefazolin in morbidly obese versus non-obese patients determined using clinical microdialysis

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Objectives: As morbidly obese patients are prone to surgical site infections, adequate blood and subcutaneous tissue concentrations of prophylactic antibiotic agents during surgery are imperative. In this study we evaluated cefazolin subcutaneous adipose tissue distribution in morbidly obese and non-obese patients, thereby quantifying the influence of morbid obesity on cefazolin pharmacokinetics and enabling Monte Carlo simulations for subsequent dose adjustments.

Methods: Nine morbidly obese patients [body mass index (BMI) 47 ± 6 kg/m²], of whom eight were evaluable, and seven non-obese patients (BMI 28 ± 3 kg/m²) received cefazolin 2 g intravenously before surgery (NCT01309152). Using microdialysis, interstitial space fluid (ISF) samples of subcutaneous adipose tissue were collected together with total and unbound plasma cefazolin samples until 240 min after dosing. Using NONMEM, population pharmacokinetic modelling, covariate analysis and Monte Carlo simulations were performed.

Results: The unbound (free) cefazolin ISF penetration ratio (\( f_{\text{AUC}_{\text{tissue}}}/f_{\text{AUC}_{\text{plasma}}} \)) was 0.70 (range 0.68–0.83) in morbidly obese patients versus 1.02 (range 0.85–1.41) in non-obese patients (\( P < 0.05 \)). A two-compartment model with saturable protein binding was identified in which the central volume of distribution and cefazolin distribution from the central compartment to the ISF compartment proved dependent on body weight (\( P < 0.001 \) and \( P < 0.01 \), respectively). Monte Carlo simulations showed reduced probability of target attainment for morbidly obese versus non-obese patients for MIC values of 2 and 4 mg/L.

Conclusions: This study shows that cefazolin tissue distribution is lower in morbidly obese patients and reduces with increasing body weight, and that dose adjustments are required in this patient group.

Keywords: subcutaneous ISF, population pharmacokinetics, Monte Carlo simulations, surgical prophylaxis

Introduction

The prevalence of obesity [body mass index (BMI) >30 kg/m²] and morbid obesity (BMI >40 kg/m²) is increasing worldwide. European obesity prevalence rates range between 4% and 37%, while in the USA 36% of the population is obese and 5% is morbidly obese.1,2 Obesity and morbid obesity are considered an independent risk factor for postoperative surgical site infection.3 To prevent surgical site infection, for surgery above or including the duodenum, cefazolin is the prophylactic agent of choice.5 As a target site for prophylactic antibiotics, distribution to the interstitial space fluid (ISF) of the subcutaneous adipose tissue should be considered. At least between opening and closure of the skin, the unbound cefazolin concentration in the ISF should be above the MIC for the target microorganisms.7

Despite extensive use of cefazolin as antibiotic prophylaxis, there are limited data available from controlled clinical trials in morbidly obese patients. Previous studies in morbidly obese patients have so far only reported cefazolin concentrations in biopsy samples taken from fat tissue, but these samples inadequately reflect unbound cefazolin concentrations in the ISF as biopsy samples provide average concentrations for combined...
intra- and extracellular compartments. Furthermore, cefazolin is highly protein bound and thus only a relatively small part of the concentration is available for antibiotic effect. To date, clinical microdialysis is the only sampling technique that allows measurement of extracellular, unbound (i.e. active) drug concentrations in virtually any tissue and is hence suitable for measuring unbound cefazolin concentrations in the ISF of the subcutaneous adipose tissue.11,12

Therefore, the objective of this study was to measure and compare unbound cefazolin concentrations in the ISF of the subcutaneous adipose tissue of morbidly obese and non-obese patients, using a microdialysis technique. The results were used to quantify the influence of overweight on cefazolin pharmacokinetics by developing a model for total and unbound plasma cefazolin and unbound cefazolin in the ISF, which can be used for Monte Carlo simulations and subsequent dose adjustments.

Materials and methods

Patients

Morbidly obese patients (BMI >40 kg/m²) undergoing laparoscopic gastric bypass surgery and non-obese patients (BMI 20–30 kg/m² at inclusion in the study) undergoing laparoscopic Toupet fundoplication surgery were considered for inclusion in the study. Patients were excluded from the study if they were pregnant, breastfeeding, suffered from renal insufficiency, had a known allergy to cefazolin or had an ejection fraction <35%. Before participation, all patients gave written informed consent. Laboratory values for evaluation of renal function were available after inclusion of the patients in the study. The study was approved by the local human research and ethics committee of Nieuwegein (VCMO), The Netherlands (NL33065.100.10) and was conducted according to the principles of the Declaration of Helsinki (version 22-10-2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO) of the Netherlands.

Study design and procedure

This was a prospective observational study (NCT01309152). For anaesthesia, all patients received propofol/remifentanil and received a 2 g intravenous (iv) bolus injection of cefazolin 15.6 ± 4.3 (range 8–24) min before the start of surgery. Up to 4 h after the cefazolin dose, blood and subcutaneous ISF samples were collected. Arterial blood samples were drawn for the measurement of total and unbound plasma cefazolin, while subcutaneous adipose ISF samples were collected using clinical microdialysis. Three hours before surgery a microdialysis probe (CMA60, Microdialysis, Solna, Sweden) was inserted in the subcutaneous tissue of the right or left side of the abdomen. After a 20 min baseline perfusion period with blank lactated Ringer’s, the catheter was perfused with 5 mg/L cefazolin in lactated Ringer’s solution for 40 min for calibration of the microdialysis catheter using the retrodialysis technique.14 A sample was collected during the calibration. At the time of cefazolin iv administration, microdialysis sample collection was started and samples were collected every 20 min until 4 h after the dose. As a result, each collected microdialysis sample represented the average concentrations over a time span of 20 min. Throughout the whole procedure the microdialysis flow rate was kept at 2 µL/min.

In non-obese patients, to determine total and unbound cefazolin concentrations in plasma, arterial blood samples were taken before and 5, 10, 30, 60, 120 and 240 min after the cefazolin iv dose. In morbidly obese patients, arterial blood samples for total cefazolin concentrations in plasma were taken before and 10, 120 and 240 min after the dose and samples for unbound plasma cefazolin were collected 5, 10, 30, 60, 120 and 240 min after the cefazolin iv dose. Blood samples were centrifuged at 3000 rpm (1500 g) for 15 min at 4°C and plasma was collected. Both plasma and microdialysis samples were stored at −80°C until analysis.

Drug assay

Total and unbound cefazolin concentrations in plasma were determined using a validated reversed-phase HPLC method with UV detection at 254 nm (total plasma cefazolin concentrations) and 272 nm (unbound cefazolin plasma and microdialysis concentrations), based on a modification of the method of Kamarni et al., described previously. In brief, a LiChrospher 100 RP-18 5 µm column was used for separation and the mobile phase, a mixture of 0.01 M acetic acid, acetonitrile and methanol (87.4/12/0.6, v/v/v), was eluted at 0.71 mL/min. Microdialysis samples were injected directly onto the HPLC column. The limit of detection and limit of quantification for unbound cefazolin concentrations in plasma and cefazolin in lactated Ringer’s (microdialysis samples) were 0.3 and 1 mg/L, respectively. For total cefazolin concentrations in plasma, the limit of detection and lower limit of quantification were 1 and 5 mg/L, respectively.

Statistical analysis

Student’s t-test was applied to test differences in demographic variables between the study groups. For cefazolin concentrations the non-parametric Mann–Whitney test was applied to test statistical differences between the groups. The observed area under the time–concentration curve from 0 to 4 h after the dose (AUC0–4 h) was calculated for each patient separately, using the linear trapezoidal rule. Outlying data were evaluated using the Grubbs’ test for detecting outliers. These statistical analyses were performed using IBM SPSS software, version 19.0.0.

Population pharmacokinetic analysis and internal validation

The population pharmacokinetic analysis was performed by means of non-linear mixed effects modelling using NONMEM (version 6.2, release 1.1; GloboMax LLC, Hanover, MD, USA). S-Plus (version 6.2; Insightful Software, Seattle, WA, USA) with NM.SP.interface version 05.03.02 (LAP&P, Leiden, the Netherlands) was used to visualize the data. Discrimination between different models was made by comparison of the objective function value (OFV, i.e. −2 log likelihood (−2LL)). A Pvalue of <0.05, representing a decrease of 3.84 in the OFV, was considered statistically significant. In addition, goodness-of-fit plots (observed versus individual-predicted concentrations, observed versus population-predicted concentrations, conditional weighted residuals versus time and conditional weighted residuals versus population-predicted concentrations plots) were used for diagnostic purposes. Furthermore, the confidence interval of the parameter estimates, the correlation matrix and visual improvement of the individual plots were used to evaluate the model. The internal validity of the population pharmacokinetic model was assessed by the bootstrap resampling method using 250 replicates and normalized prediction distribution errors (NPDEs).
Parameters obtained with the bootstrap replicates were compared with the estimates obtained from the original dataset. NPDE plots were checked for normal distribution characteristics and trends in the data errors.

**Structural model**

To describe all cefazolin concentrations (total plasma, unbound plasma and unbound subcutaneous ISF concentrations) a two-compartment model (ADVAN 6) was used. The model was parameterized in terms of the volume of distribution of the central compartment (V1), volume of distribution of the subcutaneous ISF compartment (V2), inter-compartmental volume of distribution of the central compartment (V1), volume of distribution (Q), clearance from the central compartment (CL) and the fraction clearance from the central compartment to the subcutaneous compartment (CAF). The parameters obtained with the bootstrap replicates were compared with the estimates obtained from the original dataset. NPDE plots were checked for normal distribution characteristics and trends in the data errors. Parameters obtained with the bootstrap replicates were compared with the estimates obtained from the original dataset. NPDE plots were checked for normal distribution characteristics and trends in the data errors.

**Statistical model**

The individual parameter estimate (empirical Bayes estimate or post hoc value) of the i-th individual was modelled according to (equation 3):  

$$ \theta_i = \theta_{\text{mean}} \times \exp^Y $$  

where $\theta_{\text{mean}}$ is the population mean and $\eta_i$ is a random variable for the i-th individual with a mean of zero and variance of $\omega^2$, assuming log-normal distribution in the population.

The residual variability, resulting from assay errors, model misspecifications and other unexplained sources, was best described with a proportional error model for total and unbound cefazolin plasma concentrations and a separate proportional error for unbound subcutaneous ISF cefazolin concentrations. The j-th observed cefazolin concentration of the i-th individual ($Y_{ij}$) is described by equation 4:  

$$ Y_{ij} = C_{\text{pred,ij}} \times (1 + \epsilon_{ij}) $$  

where $C_{\text{pred,ij}}$ is the population predicted cefazolin concentration in the i-th individual at the j-th time and $\epsilon_{ij}$ is a random variable with a mean of zero and variance of $\alpha^2$.

**Covariate analysis**

Covariates were plotted independently against the individual estimates of pharmacokinetic parameters to visualize potential relations. The following covariates were tested: total body weight (TBW), BMI, lean body weight (LBW), sex, obesity and age. Covariates (except for sex and obesity) were tested using linear and allometric equations (equations 5 and 6):  

$$ P_i = P_p \times \left( \frac{\text{COV}_{\text{median}}}{\text{COV}} \right)^k $$  

$$ P_i = P_p \times (1 + Y \times (\text{COV} - \text{COV}_{\text{median}}) $$  

where $P_i$ and $P_p$ represent individual and population parameter estimates, respectively; COV represents the covariate; COV$_{\text{median}}$ represents the median value of the covariate for the population; $X$ represents the exponential scaling factor, which was fixed at 1 for a linear function or an estimated value for a power function; and $Y$ represents a correlation factor between the population pharmacokinetic parameters and the change in covariate value. The binary covariates sex and obesity were tested using the following equation:  

$$ P_i = P_p \times Z^{\text{COV}} $$  

where $P_i$ and $P_p$ represent individual and population parameter estimates, $Z$ the estimated factor of increase or decrease for the patient subgroup with COV equalling 1. Potential covariates were separately entered into the model and statistically tested by use of the OFV and, if applicable, the 95% CI of the additional parameter. In addition, if applicable, we evaluated whether the interindividual variability in the parameter concerned reduced in value upon inclusion of the covariate on the parameter. When more than one significant covariate for the simple model was found, the covariate-adjusted model with the largest decrease in the OFV was chosen as a basis to sequentially explore the influence of additional covariates with the use of the same criteria. Finally, after forward inclusion ($P < 0.05$), a backward exclusion procedure was applied to justify the inclusion of a covariate ($P < 0.01$). The choice of the covariate was further evaluated as discussed above (in the Population pharmacokinetic analysis and internal validation section).

**Monte Carlo simulations**

Monte Carlo simulations based on body weight and age distributions of the original populations were performed to simulate cefazolin concentration–time profiles of 5000 morbidly obese patients and 5000 non-obese patients. In these simulations, the unbound (free) area under the curve ratios ($\text{AUC}_{\text{tissue}}/\text{AUC}_{\text{plasma}}$) were calculated by allowing the unbound plasma and subcutaneous concentrations to accumulate over time in hypothetical compartments.

**Results**

**Patients and data**

Nine morbidly obese patients with a mean body weight of 141.4 ± 22 kg (range 107 – 175) and seven non-obese patients with a mean body weight of 86.2 ± 13 kg (range 72 – 109) participated in the study. Immediately after inclusion, one morbidly obese patient was excluded from the study because of an estimated glomerular filtration rate (GFR) of 60 mL/min instead of...
an estimated GFR >60 mL/min (ID 3), which was noticed after inclusion. Furthermore, ISF measurements from another morbidly obese patient were excluded from the analysis because the unbound area under the ISF curve (\(\text{fAUC}_{\text{ISF},0-4\text{ h}}\)) and \(\text{fAUC}_{\text{tissue}}/\text{fAUC}_{\text{plasma}}\) ratio of this patient were strongly deviating and outlying based on the Grubbs’ test for detecting outliers

\[P<0.05\] and \(P<0.01\), respectively (ID 2).

Patient characteristics of eight morbidly obese and seven non-obese patients are summarized in Table 1.

### Observed cefazolin concentrations in plasma and ISF

Figure 2 shows the median and IQR of observed cefazolin concentrations for morbidly obese and non-obese patients; panel (a) shows unbound cefazolin in the ISF of the subcutaneous adipose tissue, panel (b) shows unbound plasma concentrations and panel (c) shows total plasma concentrations.

The area under the time–unbound concentration curve \(\text{fAUC}_{0-4\text{ h}}\) for subcutaneous ISF was significantly lower in morbidly obese patients \((n=7)\) in comparison with non-obese patients \((n=7), P<0.05\). In contrast, the \(\text{fAUC}_{0-4\text{ h}}\) for unbound plasma cefazolin concentrations did not differ significantly between the patient populations \((P>0.05)\). The observed unbound cefazolin ISF penetration ratio, expressed as \(\text{fAUC}_{\text{tissue}}/\text{fAUC}_{\text{plasma}}\), was 0.70 (range 0.68–0.83) in morbidly obese patients as opposed to 1.02 (range 0.85–1.41) in non-obese patients \((P<0.05)\).

### Population pharmacokinetic model and validation

A two-compartment pharmacokinetic model with saturable plasma protein binding best described the data (Figure 1, equation 2). Using this structural model without covariates, total and unbound plasma cefazolin concentrations in both patient groups were well described, while individual and population-predicted subcutaneous ISF concentrations were overpredicted in morbidly obese patients and underpredicted in the non-obese patients. Exploration and testing of covariates for \(V_1, V_2, Q, CL\) and \(B_{\text{max}}\) showed improvements of fit for unbound cefazolin plasma concentrations; however, the observed trend for subcutaneous ISF cefazolin concentrations (overprediction for morbidly obese patients, underprediction for non-obese patients) could not be explained by any of the preliminary covariates on any of the parameters. Therefore, potential non-linearity in cefazolin distribution from the central \((V_1)\) to the subcutaneous ISF compartment \((V_2)\) was evaluated by applying a power function \((\gamma)\) to the cefazolin amount (concentration) in the central compartment \((A_1)\):

\[
\Delta A_1/\Delta t = -k_{12} \times A_1^\gamma \times FU - k_{10} \times A_1 \times FU + k_{31} \times A_2
\]

where \(A_1\) is the amount of cefazolin in the \(xth\) compartment, \(FU\) is the fraction unbound (equation 2) and \(k_{12}\) is a rate constant between compartments 1 and 2.

Although no non-linearity was identified because \(\gamma\) was not found to differ significantly from 1, addition of interindividual variability in \(\gamma\) strongly improved the goodness of fit of the subcutaneous ISF concentrations \((P<0.001, -124 \Delta OFV)\). Parameter values of the simple model without covariates are summarized in Table 2.

With the extended model, a covariate analysis was performed and exploratory plots of covariates against individual post hoc parameter estimates of the simple model showed potential relationships for different body size descriptors (TBW, BMI and LBW).

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Morbidly obese</th>
<th>Range</th>
<th>Non-obese</th>
<th>Range</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female</td>
<td>1/7</td>
<td>4/3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.1 ± 5.5</td>
<td>32–48</td>
<td>53.7 ± 6.3</td>
<td>42–61</td>
<td>0.001</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>140.4 ± 23</td>
<td>107–175</td>
<td>86.2 ± 13</td>
<td>72–109</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lean body weight (kg)</td>
<td>75.2 ± 8.5</td>
<td>64–89</td>
<td>55.5 ± 5.7</td>
<td>48–62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>47.0 ± 5.8</td>
<td>41–57</td>
<td>28.2 ± 2.8</td>
<td>24–31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Surgery duration (min)</td>
<td>63.6 ± 12</td>
<td>51–86</td>
<td>59.6 ± 19</td>
<td>39–92</td>
<td>0.640</td>
</tr>
<tr>
<td>Wound closure post dose (min)</td>
<td>79.4 ± 14</td>
<td>65–105</td>
<td>74.1 ± 19</td>
<td>55–108</td>
<td>0.557</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation and range.

Figure 2. Observed cefazolin concentrations (median ± IQR) in morbidly obese (black symbols and line, \(n=7\) for plot (a), \(n=8\) for plots (b) and (c)) and non-obese (grey symbols and line, \(n=7\)) patients. (a) Subcutaneous ISF cefazolin. (b) Unbound plasma cefazolin. (c) Total plasma cefazolin.
with volume of distribution (V1) and the γ factor, for age and TBW with clearance and for LBW with B\textsubscript{max}. After forward inclusion and backward deletion of covariates in the model, TBW proved to be the strongest predictor of interindividual variability of both central volume of distribution (P < 0.001, −77 ΔOFV) and γ representing cefazolin distribution to subcutaneous tissue (P < 0.01, −10 ΔOFV). For clearance, age significantly improved the model (P < 0.01, −10 ΔOFV). Finally, LBW seemed to be a covariate for B\textsubscript{max}; however, this covariate relationship was not included in the final model due to limited statistical significance (P > 0.01, −6 ΔOFV) in the backward deletion step.

Parameter estimates of the final covariate model are summarized in Table 2. The table shows that implementation of the covariates age and TBW on the parameter, γ and clearance in the final model indeed explained variability in these parameters (decrease in interindividual variability in γ and clearance of 1.1% and 8.6%). Figure 3 shows observed versus population-predicted cefazolin concentrations in the ISF of subcutaneous tissue (b), unbound plasma (d) and total plasma (f) for morbidly obese and non-obese patients of the final model. The figure shows that there was no remaining bias in any of the plots between data from morbidly obese or non-obese patients, except for a slight overestimation of the lower subcutaneous concentrations in some of the morbidly obese patients (Figure 3b).

The final covariate model was validated using bootstrap analysis, confirming the results (Table 2), and NPDE analysis, which indicated normal distributions of errors (Figure S1, available as Supplementary data at JAC Online).

Monte Carlo simulations
The final covariate model was used to simulate concentration–time profiles of subcutaneous ISF and unbound plasma cefazolin in 5000 morbidly obese and 5000 non-obese patients. The probabilities of the patient groups remaining above a certain MIC 120, 180 and 240 min after a 2 g iv dose are summarized in Table 3. Figure 4 illustrates the probability of target attainment that can be expected for unbound cefazolin concentrations in the ISF of morbidly obese versus non-obese patients. It shows that the probabilities of target attainment of subcutaneous ISF cefazolin concentrations are lower for morbidly obese patients, while probabilities of target attainment of unbound cefazolin plasma concentrations are more similar in both patient groups. The mean simulated unbound cefazolin ISF penetration ratio, expressed as f\textsubscript{AUC\_tissue}/f\textsubscript{AUC\_plasma}, was 0.85 ± 0.19 in morbidly obese patients and 1.14 ± 0.27 in non-obese patients.

Discussion
This study aimed to measure and compare unbound cefazolin concentrations in the ISF of the subcutaneous adipose tissue of morbidly obese and non-obese patients and to quantify the influence

### Table 2. Population pharmacokinetic parameters of the simple and final pharmacokinetic model for cefazolin in morbidly obese and non-obese patients and results of bootstrap analysis (250/250 resamples successful)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Simple model (CV)</th>
<th>Final model (CV)</th>
<th>Bootstrap (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed effects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (L/min)</td>
<td>0.384 (7.5)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CL\textsubscript{47 years} \cdot {AGE/47}^{-1}</td>
<td>—</td>
<td>0.371 (5.7)</td>
<td>0.371 (5.5)</td>
</tr>
<tr>
<td>V1 (L)</td>
<td>8.79 (5.1)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>V\textsubscript{109 kg} \cdot {1+Y \cdot [TBW-109]}</td>
<td>—</td>
<td>8.94 (3.0)</td>
<td>8.97 (3.1)</td>
</tr>
<tr>
<td>V2 (L)</td>
<td>8.1 (8.9)</td>
<td>530 (7.8)</td>
<td>469 (7.3)</td>
</tr>
<tr>
<td>B\textsubscript{max} (μM)</td>
<td>530 (7.8)</td>
<td>469 (7.3)</td>
<td>471 (7.6)</td>
</tr>
<tr>
<td>K\textsubscript{d} (μM)</td>
<td>81.2 (11.4)</td>
<td>71.3 (10.9)</td>
<td>71.7 (11.2)</td>
</tr>
<tr>
<td>γ</td>
<td>1 fixed</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>γ\textsubscript{pop} \cdot {TBW/109}^2</td>
<td>1 fixed</td>
<td>1 fixed</td>
<td>1 fixed</td>
</tr>
<tr>
<td>γ\textsubscript{population}</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Z</td>
<td>−0.0946 (−27.6)</td>
<td>−0.0895 (−34.2)</td>
<td></td>
</tr>
<tr>
<td><strong>Interindividual variability (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>31.2 (28.1)</td>
<td>22.6 (31.6)</td>
<td>21.5 (32.9)</td>
</tr>
<tr>
<td>B\textsubscript{max}</td>
<td>20.6 (41.6)</td>
<td>11.6 (33.4)</td>
<td>10.8 (41.4)</td>
</tr>
<tr>
<td>γ</td>
<td>3.9 (28.9)</td>
<td>2.8 (50.4)</td>
<td>2.7 (53.2)</td>
</tr>
<tr>
<td><strong>Residual variability (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total and unbound plasma</td>
<td>13.1 (18.2)</td>
<td>10.0 (22.2)</td>
<td>9.7 (22.1)</td>
</tr>
<tr>
<td>subcutaneous ISF</td>
<td>16.5 (19.9)</td>
<td>17.0 (19.7)</td>
<td>16.7 (19.5)</td>
</tr>
<tr>
<td>OFV</td>
<td>1260.5</td>
<td>1166.5</td>
<td>1142.0 (9.7)</td>
</tr>
</tbody>
</table>

AGE, age in years; CV, coefficient of variation (%); TBW, total body weight (kg).

Cefazolin microdialysis and Pop-PK in obese and non-obese patients...
Figure 3. Observed versus individual predicted (a, c, e) and population-predicted (b, d, f) cefazolin concentrations of subcutaneous ISF cefazolin (a and b), unbound plasma cefazolin (c and d) and total plasma cefazolin (e and f) in morbidly obese and non-obese patients. The dashed line represents the line of identity ($x = y$).
of overweight and other covariates on cefazolin pharmacokinetics. Using clinical microdialysis, it was found that unbound cefazolin subcutaneous tissue penetration was lower in morbidly obese compared with non-obese patients. When analysing these results in a population analysis, a two-compartment population pharmacokinetic model with saturable protein binding was found to adequately describe all measured cefazolin concentrations. The covariate analysis showed that the central volume of distribution increased linearly with body weight and that cefazolin distribution from the central to the subcutaneous compartment decreased with body weight in a non-linear manner.

Unbound cefazolin concentrations in the ISF of subcutaneous adipose tissue have not been reported previously for morbidly obese patients, despite the fact that reduced tissue penetration of antibiotic agents in morbidly obese versus non-obese patients has been reported. Cefoxitin, which is a cephalosporin class antibiotic agent, like cefazolin, also showed reduced tissue penetration in morbidly obese versus non-obese patients (0.08 ± 0.07 versus 0.37 ± 0.26, P < 0.05), although this AUC ratio was calculated using total cefoxitin plasma concentrations instead of unbound plasma concentrations, while cefoxitin is ~34% protein bound. Also, in that study morbidly obese patients were compared with mostly healthy volunteers who did not undergo surgery. Furthermore, reduced tissue penetration in morbidly obese patients was found for ciprofloxacin (0.45 ± 0.27 versus 0.82 ± 0.36, P < 0.01), though in the study by Hollenstein et al. protein binding was not considered either. The lower drug penetration into the subcutaneous adipose tissue of morbidly obese patients found in these studies and in the present study may potentially be explained by lower subcutaneous adipose tissue blood flow. It has been shown before that subcutaneous adipose tissue blood flow in obese and morbidly obese patients is lower than in healthy control subjects. Additionally, Joukhadar et al. found in healthy volunteers that enhanced subcutaneous blood flow resulted in higher subcutaneous ciprofloxacin concentrations. Therefore, we think that the lower subcutaneous adipose tissue penetration of cefazolin in morbidly obese patients after a single dose may be explained by lower subcutaneous adipose tissue blood flow.

### Table 3. Probability of 5000 Monte Carlo-simulated morbidly obese and 5000 non-obese patients attaining cefazolin MIC targets of 1, 2 and 4 mg/L 120, 180 and 240 min after a 2 g iv cefazolin dose

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>120 min post dose</th>
<th>180 min post dose</th>
<th>240 min post dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morbidly obese</td>
<td>Non-obese</td>
<td>Morbidly obese</td>
</tr>
<tr>
<td>&gt;1 mg/L</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt;2 mg/L</td>
<td>1.00</td>
<td>1.00</td>
<td>0.996</td>
</tr>
<tr>
<td>&gt;4 mg/L</td>
<td>0.996</td>
<td>1.00</td>
<td>0.909</td>
</tr>
</tbody>
</table>

### Figure 4. Probability of target attainment (PTA) at four different MIC values 120, 180 and 240 min after a 2 g cefazolin iv dose in 5000 morbidly obese and 5000 non-obese Monte Carlo-simulated patients. (a) PTA of unbound cefazolin concentrations in the subcutaneous ISF. (b) PTA of unbound plasma cefazolin concentrations.
In the population pharmacokinetic model the difference in subcutaneous ISF cefazolin concentrations between morbidly obese and non-obese patients was not adequately described by the effect of TBW on central volume of distribution (V1) alone or by additional covariates for intercompartmental clearance (Q) or the subcutaneous ISF compartment (V2). However, the introduction of interindividual variability on a γ factor (equation 8) on the distribution of cefazolin amount from the central (V1) to the subcutaneous compartment (V2) was able to improve the goodness of fit of the subcutaneous cefazolin data for both patients groups. Despite the small absolute difference in γ between a morbidly obese and non-obese patient, it strongly impacts on cefazolin distribution from the central to the subcutaneous compartment: where a non-obese individual of 75 kg with a corresponding γ value of 1.02 transports 300 mg unbound cefazolin/min from the central to the subcutaneous compartment, a morbidly obese patient of 145 kg with a corresponding γ value of 0.96 transports only 210 mg/min (30% difference). For the final pharmacokinetic model, TBW on V1 and TBW on the γ factor were found to be the most predictive covariates for the reduced cefazolin distribution observed in morbidly obese patients. The slight overestimation of lower subcutaneous cefazolin concentrations in morbidly obese patients can be explained by the relatively high interindividual variability observed for cefazolin subcutaneous concentrations. While the model underpredicts concentrations 230 min after dosing for some morbidly obese patients, for others it overestimates concentrations at the same time after the dose.

In contrast to the differences observed in cefazolin distribution between morbidly obese and non-obese patients, we found that cefazolin saturable protein binding was similar for both patient groups. Plasma albumin concentrations were not measured in this study and may have been a covariate for maximal binding capacity (B_{max}). However, for this parameter interindividual variability was relatively small (11.6%) and thus the influence of a difference in albumin concentration on cefazolin pharmacokinetics is assumed to be limited. Furthermore, the extent of saturable protein binding corresponded to earlier reports in non-obese and morbidly obese patients, and estimated B_{max} and K_d values correspond to values found in earlier studies in human plasma, in which B_{max} was reported to be 438 μM and K_d was 50 and 60.2 μM.\textsuperscript{29,30}

To determine the efficacy of prophylactic cefazolin, currently the time of unbound plasma cefazolin above the MIC (T_{>\text{MIC}}) between opening and closure of the wound is used as the pharmacokinetic/pharmacodynamic (PK/PD) index. However, this is based on the assumption that cefazolin penetration from plasma to the ISF of the subcutaneous tissue is equal to 1,\textsuperscript{7} whereas in this study it was found that cefazolin tissue distribution was lower than 1 for morbidly obese patients. This suggests that for morbidly obese patients ISF tissue concentrations rather than unbound plasma concentrations should be considered as the PK/PD index to target for cefazolin efficacy. Monte Carlo simulations allowed evaluation of cefazolin ISF tissue concentrations in large simulated patient populations and indicated that a dose of 2 g iv cefazolin given prior to incision will be sufficient to prevent wound infections with pathogens for which the MIC is 1 mg/L in a 120 min surgical procedure. However, when higher MIC values apply (e.g. 2 or 4 mg/L) redosing may be required after 2 h as the probability of attaining a target of 4 mg/L at 180 min post dose had dropped to 0.909 for morbidly obese as opposed to 0.995 for non-obese patients, while for a target of 2 mg/L the probability of target attainment was 0.956 in morbidly obese versus 0.997 in non-obese patients at 240 min post dose (Table 3). Alternatively, it is obvious that if surgery is prolonged beyond 4 h, an extra dose is necessary even when an MIC of 1 mg/L is taken as the reference value.

The design of the current study allowed a straightforward and extensive comparison of unbound cefazolin concentrations in both plasma and ISF of the subcutaneous adipose tissue in morbidly obese and non-obese patients undergoing laparoscopic gastric surgery. In addition, it allowed a quantitative analysis of the influence of morbid obesity on cefazolin distribution. Nevertheless, the current study has some limitations. Firstly, this study only included 15 patients, which may limit the accuracy of estimation of interindividual and residual variability of pharmacokinetic parameters, which in turn may prevent broad conclusions being drawn regarding cefazolin efficacy in morbidly obese patients. Also, extrapolation of this model to patients beyond the body weight ranges of these data should be exercised with caution. However, the data gathered in this study are rather unique in terms of both methods (rich data, semi-simultaneous observations in ISF and plasma) and patients, and currently no other evidence about cefazolin efficacy in morbidly obese patients is available. Secondly, the ISF data from one morbidly obese patient was excluded from the pharmacokinetic analysis, because the ISF time–concentration profile of this patient was highly deviating and outlying in comparison with the other morbidly obese patients in this study. Deviation may be explained by the relatively low microdialysis recovery ratio measured for this patient (13.6%, compared with a mean of 28.1 ± 7.9%). Thirdly, it should be stated that the model developed here slightly overestimates the lower subcutaneous cefazolin concentrations in some of the morbidly obese patients. If the model had predicted these lower cefazolin ISF concentrations more accurately, the probability of target attainment results from the Monte Carlo simulation may have been even more disadvantageous for morbidly obese patients. Finally, it is assumed that these potential weaknesses do not explain the lower cefazolin tissue penetration found for morbidly obese patients in this study.

In conclusion, this study showed that cefazolin distribution to the ISF of the subcutaneous adipose tissue is reduced in morbidly obese versus non-obese patients, that cefazolin tissue distribution decreases with increasing body weight and that dose adjustments are required in this patient group.

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Cefazolin microdialysis and Pop-PK in obese and non-obese

Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


