Blood, tissue and intracellular concentrations of Erythromycin and its metabolite anhydro-erythromycin during and after end of therapy

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Running title: Plasma, tissue and cellular PK of erythromycin

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Abstract

For macrolides clinical activity but also the development of bacterial resistance have been attributed to prolonged therapeutic and sub-therapeutic concentrations. Although erythromycin is a long established antimicrobial, concomitant determination of the pharmacokinetics of erythromycin and its metabolites in different compartments is limited. To better characterize pharmacokinetics of erythromycin and its anhydro-metabolite (AHE) in different compartments during and after end of treatment with 500 mg erythromycin four times daily, concentration-time profiles were determined in plasma, interstitial space of muscle and subcutaneous adipose tissue, and white blood cells (WBC) at day 1 and 3 of treatment and 2 and 7 days after end of therapy. In WBC concentrations of erythromycin exceeded those in plasma approximately 40-fold, while free concentrations in plasma and tissue were comparable. The observed delay of peak concentrations in tissue might be caused by fast initial cellular uptake. Two days after the end of treatment sub-inhibitory concentrations were observed in plasma and interstitial space of both soft tissues, while 7 days after end of treatment erythromycin was not detectable in any compartment. This relatively short period of sub-inhibitory concentrations may be advantageous compared to other macrolides. The ratio of erythromycin over AHE on day 1 was highest in plasma (2.81 ± 3.45) and lowest in WBC (0.27 ± 0.22). While the ratio remained constant between single dose and steady state, after end of treatment the concentration of AHE declined slower than that of the parent compound, indicating the importance of the metabolite for prolonged drug interaction of erythromycin.
Macrolides account for 10-15% of the worldwide consumption of antibiotics. (25, 31) Although in many countries erythromycin was replaced by newer relatives like azithromycin and clarithromycin, prescription of erythromycin still has a substantial place in less developed countries but also individual regions within Europe. (15, 40) However, bacterial resistance against macrolides dramatically escalated in many countries during the last decades. (14, 23, 35) Total consumption of antimicrobials on the one hand and the presence of long periods of exposure of bacterial populations to sub-inhibitory concentrations of antibiotics on the other hand are considered critical factors for selecting bacterial resistance. (2, 3, 16)

Erythromycin is known for its highly variable bioavailability after oral ingestion and its susceptibility towards degradation under acidic conditions. Although its half live in plasma is short and the pharmacokinetic (PK) profile intra-individually heterogeneous it is very well distributed throughout body tissues and accumulates in leucocytes. (11) While clinical studies have shown the effectiveness of erythromycin in skin and soft tissue infections, the in vivo penetration and the resulting free concentrations in interstitial space of soft tissues, i.e. the most important site of bacterial infections, have not been reported until now. (33, 39) High cellular uptake, relatively short retention and fast back release from white blood cells (WBC) are considered important properties of the PK of erythromycin. (1) For macrolides, intracellular accumulation was considered to prolong their antibacterial effects. (6) However, a clearer picture of the PK of erythromycin in the totality of different compartment seems necessary to understand the complex pharmacokinetic – pharmacodynamic (PK/PD) interactions of macrolides with regard to antimicrobial effects as well as development of bacterial resistance.
Erythromycin is hydrolyzed to anhydro-forms (anhydro-erythromycin (AHE) and other metabolites) and this process is potentiated by acidic conditions. AHE is microbiologically inactive but inhibits drug oxidation in the liver and thus is considered to be an important factor for the unwanted drug-drug interactions of erythromycin. In vitro AHE is a more potent inhibitor of the cytochrome P450 3A subfamily than erythromycin, thereby prolonging elimination of benzodiazepines, immunosuppressants, statins and many more drugs. Acidic conditions in stomach increase the level of AHE but high levels of AHE were also found in inflamed tonsils.

Thus, due to the lack of currently available information on the target site penetration of erythromycin and its main metabolite, we for the first time determined the concentration-time profiles of erythromycin and AHE simultaneously in the interstitial space fluid of muscle, subcutaneous adipose tissue and in leucocytes. We compared PK parameters of those compartments with plasma concentrations during 3 days of active treatment and after the end of the active treatment period up to 7 days post last drug administration.
Material and methods

Regulatory issues:
This was prospective, open-label PK study (EUDRACT 2009-015678-37), conducted at the Department of Clinical Pharmacology at the General Hospital of Vienna, and performed in accordance with the actual ICH - GCP guidelines and the Declaration of Helsinki. The clinical study was approved by the Ethical Committee of Medical University of Vienna (EK Nr. 767/209) and was authorized by the Austrian Agency for Health and Food Safety (AGES).

Subjects:
Within two weeks of the start of the study volunteers underwent a screening visit including a physical examination, blood samples (hematology, clinical chemistry, virology and coagulation test), ECG and non-invasive arterial pressure in order to identify 6 eligible subjects. Key inclusion criteria were: male, age between 18-50 years, body mass index between 18 and 30, no regular concomitant medication within the last 2 weeks prior to the study day and written informed consent given by volunteers. Key exclusion criteria were known allergy or hypersensitivity against erythromycin and clinically relevant abnormal physical or laboratory findings.

Study days:
Starting with study day 1 subjects received 500 mg of erythromycin (Erythromycinethylsuccinat) four times daily (EryHexal®, Hexal AG, Germany) for three consecutive days. Erythromycin had to be taken fastened, i.e. at least 2 hours after last intake of food and 1 hour before intake of food. PK sampling was performed on study day 1 and day 3 (i.e. after a single dose and at steady state) and on study days 5 and 10 (i.e. two or seven days after end of therapy) for plasma, muscle tissue,
subcutaneous adipose tissue and WBC as described below. Subjects were confined at the department of clinical pharmacology for the duration of PK sampling on the respective study days. On study day 5 and 10 microdialysis was only performed in three subjects each (subjects were randomly assigned to undergo microdialysis on study day 5 or 10). All samples were snap frozen at approximately -20°C. Thereafter, samples were stored at approximately -80°C until analysis.

**Plasma collection:**

4.5 ml of blood was drawn from an antecubital vein for determination of erythromycin concentrations in plasma at baseline, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6 and 8 hours after drug administration on day 1 and 3, and three times on day 5 and 10. Blood samples were kept on ice for a maximum of 60 min and were centrifuged at +4°C and 4020g for 10 min to obtained plasma.

**Determination of Erythromycin in muscle tissue and subcutis:**

*In vivo* microdialysis perfusion technique was performed as previously described. (28, 29, 37, 43, 45) Microdialysis is based on sampling of analytes from the extracellular space by a semi-permeable membrane at the tip of a microdialysis probe. Once the probe is implanted into the tissue, it is constantly perfused at a low flow-rate. Substances present in the extracellular fluid diffuse from the extracellular fluid into the dialysate.

On study days two microdialysis probes (CMA63®, CMA/Microdialysis AB, Solna, Sweden) were aseptically inserted into skeletal muscle and subcutaneous adipose tissue of the thigh by use of a steel guidance split cannula. The probes then were perfused with saline solution (NaCl 0.9%, “Meditrade”, Austria) at a flow rate of 1.5
µl/min by a microinfusion-pump (CMA 107). After 30 minutes for equilibration of
tissue trauma on day 1 and 3 baseline sampling was performed for 30 minutes and
then sampling was performed in 30 minutes intervals up to three hours followed by
hourly intervals up to 10 hours after the morning dose of erythromycin. On day 5 and
10 microdialysis sampling was performed for two 2 hour intervals. At the end of each
microdialysis sampling period the calibration was performed according to the
retrodialysis method.

The principle of retrodialysis relies on the fact that the diffusion process through the
semi-permeable membrane is quantitatively equal in both directions. The \textit{in-vivo}
recovery values were thus calculated from:

\[ \text{In-vivo recovery (\%)} = 100 - \left( \frac{100 \times \text{analyte concentration out}}{\text{analyte concentration in}} \right) \]

Interstitial concentrations were calculated according to the following equation:

\[ \text{Interstitial concentration} = 100 \times \left( \frac{\text{sample concentration}}{\text{in - vivo recovery}} \right) \]

Microdialysis probes were removed at the end of each study day.

\textbf{Isolation of WBC}:

Isolation of WBC was performed using the Polymorphprep\textsuperscript{TM} (Axis-Shiled PoC AS, Norway) according to manufactures instruction at baseline, 2, 6 and 10 hours after
the morning dose of erythromycin on day 1 and 3 and once on day 5 and 10. For
each sample determination of WBC count was performed by hematology analyzer
(Sysmex analyzer CE Series 2100, United States). To obtain the average
concentration of erythromycin and its metabolite in WBC, the concentration
measured in the sample was subsequently corrected for the total cell count and the cell volume as previously described. (30)

Analytical methods:

Sample preparation

Human plasma:

All sample handling was done at +4 °C and day-light protection. Human plasma samples (100 µL) were stabilized by 0.05 mL ammonium acetate buffer (50 mM) and were deproteinized by addition of 250 µL of acetonitrile containing the internal standard. After thorough mixing, the samples were centrifuged at 11.000 rpm for 5 min at approximately +4 °C. Following centrifugation and dilution of the supernatant with 5 mM ammonium acetate buffer 20 µL of each sample were injected.

Microdialysate:

All sample handling was done at +4 °C and day-light protection. Human microdialysate samples (10 µL) were diluted by 90 µL ammonium acetate buffer (5 mM) containing the corresponding internal standard. After thorough mixing 10 µL of each sample were injected. Samples with concentration above the upper quantification limit were pre-diluted with isotonic saline solution.

Liquid chromatography/mass spectrometry

The liquid chromatography system consisted of a binary HPLC-Pump 1200 Series from Agilent Technologies (Waldbronn, Germany). Detection was performed using an AB SCIEX API 5000™ triple quadrupole mass spectrometer (AB SCIEX, Concord, Ontario, Canada; in Germany supplied by: AB SCIEX Germany GmbH, Darmstadt)
with TurboIonSpray® interface. The Analyst Version 1.4.2 (AB SCIEX Germany GmbH, Darmstadt, Germany) was used for evaluation of chromatograms. The internal standard was oleandomycin.

Each sample was chromatographed on a reversed-phase column eluted with an isocratic solvent system consisting of buffer and acetonitrile (50:50, v:v) and monitored by LC-MS/MS with a SRM method as follows: Precursor → product ion for erythromycin m/z 734.3 → m/z 158.4, anhydro-erythromycin m/z 716.5 → m/z 158.4 and for the internal standard m/z 688.2 → m/z 158.4, all analyses were in positive mode. Under these conditions erythromycin, anhydro-erythromycin and the internal standard were eluted after approximately 1.4 min, 1.5 min and 1.3 min, respectively.

Plasma samples were measured against a calibration curve prepared in the human drug free plasma. Calibration standards were prepared by adding the defined amounts of standard solution of analyte to drug free plasma. Samples were measured against a calibration curve prepared in 0.9 % NaCl. Calibration standards were prepared by adding the defined amounts of standard solution of analyte to 0.9 % NaCl. Calibration was performed by weighted (1/concentration²) linear regression.

Spiked quality controls (SQC) were prepared for determination of inter-assay variation by the addition of defined amounts of the stock solution of the analyte or the spiked control of higher concentration to defined amounts of tested drug-free human plasma, 0.9 % NaCl or human dialysate.

**Human plasma:**

The limit of quantification (LOQ) of erythromycin A and anhydro-erythromycin A in
human plasma was set to 4.00 and 4.02 ng/mL, respectively. The linearity (coefficient of correlation) was 4.00-2000 ng/mL (1.000) for erythromycin A and 4.02-2006 ng/mL (≥0.995) for anhydro-erythromycin A. The intra-day precision (accuracy) for erythromycin A and anhydro-erythromycin A in human plasma were determined as 0.8-4.6 % (98.9-100.5 %) and 2.2-5.9 % (99.0-104.8 %), respectively. The inter-day precision (accuracy) for erythromycin A and anhydro-erythromycin A in human plasma were determined as 2.5-3.5 % (99.3-100.4 %) and 6.0-8.7 % (98.3-102.0 %), respectively.

Microdialysate:

The linearity of erythromycin A in 0.9 % NaCl was shown to be between 4.00-2000 ng/mL (LOQ: 4.00 ng/mL) for the analyses of the muscle retro dialysis samples and between 1.00-500 ng/mL (LOQ: 1.00 ng/mL) for the analyses of the muscle dialysis and subcutis dialysis and retro dialysis samples and for the analyses of the leucocytes between 1.50-500 ng/mL (LOQ: 1.50 ng/mL).

The linearity of anhydro-erythromycin A in 0.9 % NaCl was shown to be between 4.01-2006 ng/mL (LOQ: 4.01 ng/mL) for the analyses of the muscle retro dialysis samples and between 1.00-502 ng/mL (LOQ: 1.00 ng/mL) for the analyses of the muscle dialysis and subcutis dialysis and retro dialysis samples and for the analyses of the leucocytes between 2.01-502 ng/mL (LOQ: 2.01 ng/mL).

The intra-day precision (accuracy) for erythromycin A in 0.9 % NaCl, muscle retro dialysis, muscle dialysis and subcutis dialysis as well as in leucocytes were.
determined as 4.7-9.5 % (99.8-104.1 %), 4.7 % (100.3 %), 5.5 % (99.6 %) and 1.7 % (102.0 %), respectively. The intra-day precision (accuracy) for anhydro-erythromycin A in 0.9 % NaCl, muscle retro dialysis, muscle dialysis and subcutis dialysis as well as leucocytes were determined as 1.4-8.2 % (92.3-107.2 %), 2.4 % (99.9 %), 7.7 % (102.2 %) and 3.0 % (100.4 %), respectively.

The inter-day precision (accuracy) for erythromycin A in 0.9 % NaCl, muscle retro dialysis and muscle dialysis and subcutis dialysis were determined as 6.3-8.4 % (97.4-106.4 %), 7.9 % (100.1 %) and 6.7 % (103.9 %), respectively. The inter-day precision (accuracy) for anhydro-erythromycin A in 0.9 % NaCl, muscle retro dialysis and muscle dialysis and subcutis dialysis were determined as 7.2-8.9 % (93.7-102.5 %), 7.0 % (98.6 %) and 6.9 % (101.2 %), respectively.

**PK and statistical analyses:**

PK parameters were calculated using a commercially available computer program (Kinetika® 3.0, Innaphase, USA): maximum concentration (C_{max}), time to maximum concentration (t_{max}), terminal elimination half-life (t_{1/2}), area under the concentration-time curve (AUC) for the dosing period of 6 h (AUC_{0-6}), AUC_{0-24}, and the total AUC (AUC_{0-\infty}) were calculated. The AUC values were calculated from non-fitted data by employing the trapezoidal rule while, while AUC_{0-24} was calculated from AUC_{0-6} multiplied by 4 for day 1 and 3 and by multiplying C_{average} by 24 for day 5. C_{average} was defined as the average concentration observed at the three sampling points on day 5. For plasma in addition apparent total body clearance (CL) and apparent volume of distribution (Vd) were calculated. Vd was based on the dose corrected for bioavailability divided by the product of AUC_{0-infinity} and the elimination constant.
obtained from plasma. AUC₀−₂₄/MIC was calculated for relevant pathogens. The free fraction of erythromycin in plasma was estimated to be 10% based on data derived from the literature. (41)

Statistical analysis was performed using SPSS® 16.0 (IBM, USA). For comparison of parameters between compartments Wilcoxon matched pairs test was employed. All data are presented as means ± standard deviation.
Results

Six healthy male volunteers (age 29.17 ± 7.68 years; weight 72.48 ± 11.49 kg; height 179.17 ± 4.02 cm and BMI 22.57 ± 3.23 kg/m²) were included in the study. Erythromycin was well tolerated and all six subjects completed the study according to the protocol. Gastric pain, pain at microdialysis probe insertion, loss of appetite, meteorism, leucocytopenia and thrombocytopenia were observed in one subject each; all adverse events were considered mild and no serious adverse events were reported.

The comparative mean concentration-time profiles of erythromycin during one dosing interval of 6 hours are shown in figure 1a and 1b for day 1 and day 3, respectively, for different compartments, including the calculated free fraction in plasma. For all compartments higher mean concentrations of erythromycin were observed on day 3 of active treatment in comparison to day 1. Two days after end of treatment a significant decrease of mean erythromycin concentrations was present, while after 7 days the erythromycin concentration was under the limit of detection in all compartments. The descriptive PK parameters of erythromycin on day 1, 3 and 5 of the study are presented in table 1, 2 and 3, respectively. In WBC concentrations of erythromycin were significantly higher than in the other compartments and exceeded concentrations in plasma approximately 40-fold. While total concentrations in plasma exceeded concentrations in interstitial space of soft tissues by approximately ten-fold, the free fraction of erythromycin in plasma was in good agreement with concentrations in muscle and subcutaneous adipose tissue.

Figure 2 shows concentration-time profile of the metabolite AHE. Although overall PK profiles of parent compound and metabolite (figure 1 and 2, respectively) looked very
similar, the ratio of both compounds differed significantly during and after end of

treatment. On day 1 the ratio of erythromycin over AHE on was highest in plasma

(2.81 ± 3.45), around 1 in interstitial space of muscle and subcutaneous adipose
tissue and lowest in WBC (0.27 ± 0.22) (figure 3). While the ratio remained relatively
constant between single dose and steady state, the proportion of AHE gets higher
after end of treatment.

The PK-PD parameter AUC0-24h/MIC was used to estimate the clinical efficacy of
erthyromycin on day 1 and day 3 of treatment as well as two days after end of
treatment against example pathogens with MIC90 of 0.125 µg/mL (erythromycin
susceptible *Streptococcus pneumoniae* or *S. pyogenes*) (Table 4a) and MIC90 of 0.5
µg/mL (erythromycin susceptible *Staphylococcus aureus* or coagulase-negative
*Staphylococcus spp.*) (Table 4b). (18)
Discussion

During the last decades PK/PD considerations have gained a central role in optimizing of clinical efficacy of antibiotic therapies and in preventing bacterial resistance. (6, 8, 27, 34) While for erythromycin various studies have determined its concentrations in blood including concentrations in WBC, until now no study has investigated the PK in the interstitial space fluid of soft tissues, perhaps the most important compartment of bacterial infections. (7, 32)

In the present study, for all compartments, mean $C_{\text{max}}$ and AUC values were significantly higher at steady state than after the first dose. However, while AUC$_{0-6}$ values for interstitial space fluid of muscle and subcutaneous adipose tissue increased more than 4-fold, the concentration in WBC increased only by the factor 1.6. Based on these data one might speculate that erythromycin first rapidly penetrates into leucocytes and later, when the accumulation capacity of WBC is saturated, the extracellular concentration increases. Indeed saturable uptake of erythromycin by WBC was previously described. (10) In line with previous in vitro experiments, after the end of therapy a fast decrease of concentrations was observed for all compartments including WBC in the present study. (24) 7 days after end of treatment erythromycin concentrations were below the limit of detection in all compartments including WBC, suggesting that no strong intracellular binding of erythromycin takes place. (10)

In-vitro cellular uptake of erythromycin was previously extensively investigated. (5, 24, 42) Active and passive transport through cell membranes resulting in accumulation of erythromycin in the intracellular compartment has been described. (42) The cellular/extracellular ratios were constant over a wide range of
concentrations. (24) Independent on the cell type antimicrobial active erythromycin rapidly effused when cells were incubated in antibiotic-free medium.

Thus, the observed accumulation of erythromycin of approximately 40-fold compared to plasma is in good agreement with previous data. (22, 26) However, a limitation of the present study is that all investigations were performed in healthy, non-inflammed tissues. Inflammation and the associated low pH might lead to reduced uptake of erythromycin into WBC, resulting in higher extracellular concentrations. (19) On the other hand acidification leads to a striking reduction of antimicrobial activity of erythromycin, so the overall impact of major local inflammation remains unclear. (17)

Acidification is also associated with acceleration of the metabolism of erythromycin into inactive metabolites like AHE. (4) AHE is microbiologically inactive but seems competent for modulating inflammation and may have other biological effects, including a potent role as inhibitor of oxidative metabolism. (36) For many inflammatory airway diseases like diffuse panbronchiolitis or cystic fibrosis, the immune-modulatory effect of erythromycin is considered responsible for beneficial effects rather than its antimicrobial action. (12) The ratio of erythromycin over AHE on day 1 was >1 in plasma, around 1 in muscle and subcutaneous adipose tissue and <1 in WBC (figure 3). During treatment almost 4-fold higher concentrations of AHE compared to parent erythromycin was found in WBC, possibly due to enhanced intracellular formation of AHE in acidic endosomal vesicles like lysosomes. While the ratio remained relatively constant between single dose and steady state, after the end of therapy absolute concentrations of AHE declined slower than concentrations of the parent compound, indicating a role of the metabolite for prolonged drug interaction.
For macrolides time above MIC or AUC\textsubscript{0-24}/MIC predict antimicrobial activity. (13, 34, 38, 44) Based on animal data bacteriostatic effects of erythromycin can be expected for free AUC\textsubscript{0-24}/MIC-ratios in plasma greater than 20, however, this value is rarely achieved in clinical practice. (9) In the present study we calculated AUC\textsubscript{0-24}/MIC-ratio for relevant pathogens like the highly susceptible \textit{S. pneumoniae} or \textit{S. pyogenes} and moderately susceptible \textit{Staphylococcus} spp. (Table 4). (18) For WBC the AUC\textsubscript{0-24}/MIC-ratios easily exceeded the value of 20 from the beginning up to two days after end of treatment for all chosen pathogens. However, for other compartments the threshold of approximately 20 could not be achieved for pathogens with an MIC of 0.5 mg/L on any study day and was only reached at day 3 of treatment for MIC values of 0.125 mg/L. It has to be noted the observed thresholds were obtained by calculations from plasma and therefore may not be readily transferred to other compartments.

Overall, for day 3 considerably better AUC\textsubscript{0-24}/MIC-ratios were achieved than on the first day of treatment. Overcoming the initially low concentrations in tissues by a loading dose, leading to a more rapid saturation of erythromycin uptake into WBC, might be an interesting option for a drug like erythromycin with good clinical tolerability. Two days after end of treatment we detected sub-inhibitory concentration in all compartments except for WBC, however, the sub-inhibitory concentrations disappeared before day 10. Compared to newer macrolides the relatively short period of sub-inhibitory concentrations may be advantageous. (23)

In conclusion, the present study confirmed the previously described fast uptake of erythromycin into WBC but additionally for the first time demonstrated that sufficient
levels of free erythromycin might be delayed until the accumulation in WBC is widely saturated. Higher initial doses of erythromycin might be considered to accelerate sufficient free concentrations in tissue. Despite this accumulation in WBC sub-inhibitory concentrations of erythromycin in the human body seem to persist relatively short. Higher concentrations of the metabolite AHE compared to parent erythromycin might be responsible for prolonged drug interactions after the end of treatment.
References:


Figure Legends:

Figure 1: comparative mean (SD) time concentration profiles of erythromycin in different compartments on a) day 1 and b) day 3 for plasma (square), the calculated free fraction in plasma (open square), muscle (up triangle), subcutaneous adipose tissue (down triangles) and WBC (diamonds) during one dosing interval of 6 hours.

Figure 2: comparative mean (SD) time concentration profiles of the erythromycin metabolite AHE on a) day 1 and b) day 3 for plasma (square), muscle (up triangle), subcutaneous adipose tissue (down triangles) and WBC (diamonds) during one dosing interval of 6 hours.

Figure 3: mean (SD) ratio of erythromycin parent compound over the metabolite AHE during day 1, day 3 for and day 5 for plasma (square), muscle (up triangle), subcutaneous adipose tissue (down triangles) and WBC (diamonds).
Table 1: Pharmacokinetic parameters of erythromycin at day 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>Tmax (h)</th>
<th>AUC&lt;sub&gt;0-6h&lt;/sub&gt; (ng*h/ml)</th>
<th>AUC&lt;sub&gt;total&lt;/sub&gt; (ng*h/ml)</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>CL (L/h)</th>
<th>VD (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Mean: 1371 SD: 653</td>
<td>Mean: 1.75 SD: 0.61</td>
<td>Mean: 3092 SD: 1150</td>
<td>Mean: 3549 SD: 1377</td>
<td>Mean: 1.67 SD: 0.25</td>
<td>Mean: 58.9 SD: 45.5</td>
<td>Mean: 135 SD: 90.5</td>
</tr>
<tr>
<td>Muscle</td>
<td>Mean: 41.9 SD: 27.3</td>
<td>Mean: 3.13 SD: 0.88</td>
<td>Mean: 129 SD: 84.2</td>
<td>Mean: 266 SD: 155</td>
<td>Mean: 3.68 SD: 1.43</td>
<td>Mean: ND SD: ND</td>
<td>Mean: ND SD: ND</td>
</tr>
<tr>
<td>Subcutis</td>
<td>Mean: 55.5 SD: 23.9</td>
<td>Mean: 2.50 SD: 0.27</td>
<td>Mean: 147 SD: 56.6</td>
<td>Mean: 218 SD: 123</td>
<td>Mean: 1.98 SD: 0.87</td>
<td>Mean: ND SD: ND</td>
<td>Mean: ND SD: ND</td>
</tr>
<tr>
<td>WBC</td>
<td>Mean: 38542 SD: 32402</td>
<td>Mean: 2.00 SD: 0.00</td>
<td>Mean: 116858 SD: 74953</td>
<td>Mean: 189846 SD: 138843</td>
<td>Mean: 3.49 SD: 3.59</td>
<td>Mean: ND SD: ND</td>
<td>Mean: ND SD: ND</td>
</tr>
</tbody>
</table>

C<sub>max</sub>: maximum concentration; Tmax: time to maximum concentration; AUC: area under the concentration time curve; T<sub>1/2</sub>: terminal elimination half-life; CL: total body clearance; Vd: apparent volume of distribution
Table 2: Pharmacokinetic parameters of erythromycin at day 3

<table>
<thead>
<tr>
<th></th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>AUC$_{0-6\text{h}}$ (ng*h/ml)</th>
<th>AUC$_{\text{total}}$ (ng*h/ml)</th>
<th>$T_{1/2}$ (h)</th>
<th>CL (L/h)</th>
<th>VD (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Mean: 2544, SD: 1266</td>
<td>Mean: 2.17, SD: 0.52</td>
<td>Mean: 8537, SD: 5419</td>
<td>Mean: 11782, SD: 9811</td>
<td>2.26, 0.84</td>
<td>18.9, 8.47</td>
<td>53.9, 17.8</td>
</tr>
<tr>
<td>Muscle</td>
<td>154, SD: 86.6</td>
<td>2.88, 1.58</td>
<td>560, 369</td>
<td>1158, 835</td>
<td>4.13, 1.11</td>
<td>ND, ND</td>
<td>ND, ND</td>
</tr>
<tr>
<td>Subcutis</td>
<td>209, SD: 139</td>
<td>2.67, 0.72</td>
<td>682, 465</td>
<td>1026, 543</td>
<td>2.85, 1.28</td>
<td>ND, ND</td>
<td>ND, ND</td>
</tr>
<tr>
<td>WBC</td>
<td>51120, SD: 26925</td>
<td>2.00, 0.00</td>
<td>190440, 101452</td>
<td>ND, ND</td>
<td>ND, ND</td>
<td>ND, ND</td>
<td>ND, ND</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$: maximum concentration; $T_{\text{max}}$: time to maximum concentration; AUC: area under the concentration time curve; $T_{1/2}$: terminal elimination half-life; CL: total body clearance; Vd: apparent volume of distribution
Table 3: Pharmacokinetic parameters of erythromycin at day 5

<table>
<thead>
<tr>
<th></th>
<th>C_{average} (ng/ml)</th>
<th>AUC_{0-24h} (ng*h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Plasma</td>
<td>34.2</td>
<td>54.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>3.58</td>
<td>6.20</td>
</tr>
<tr>
<td>Subcutis</td>
<td>5.11</td>
<td>7.99</td>
</tr>
<tr>
<td>WBC</td>
<td>7321</td>
<td>7928</td>
</tr>
</tbody>
</table>

C_{average}: average concentration on day 5; AUC: area under the concentration time curve
Table 4a: AUC\textsubscript{0-24h}/MIC of erythromycin for the example 0.125 µg/mL (MIC\textsubscript{90} of erythromycin susceptible *S. pneumoniae* and erythromycin susceptible *S. pyogenes*)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma\textsuperscript{1}</td>
<td>9.90</td>
<td>4.13</td>
<td>3739</td>
</tr>
<tr>
<td>Subcutis</td>
<td>4.71</td>
<td>27.3</td>
<td>6094</td>
</tr>
<tr>
<td>Muscle</td>
<td>37.9</td>
<td>17.9</td>
<td>0.82</td>
</tr>
<tr>
<td>WBC</td>
<td>33.9</td>
<td>21.8</td>
<td>0.71</td>
</tr>
</tbody>
</table>

\textsuperscript{1} the calculated free fraction in plasma was used.
Table 4b: AUC$_{0-24h}$/MIC of erythromycin for the example 0.5 µg/mL (MIC$_{90}$ of erythromycin susceptible *S. aureus* and erythromycin susceptible coagulase-negative *Staphylococcus spp.*)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma$^1$</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Subcutis</td>
<td>2.47</td>
<td>0.92</td>
<td>1.03</td>
</tr>
<tr>
<td>Muscle</td>
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<td>600</td>
<td>6.83</td>
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<tr>
<td>WBC</td>
<td>5.46</td>
<td>2.95</td>
<td>4.48</td>
</tr>
<tr>
<td>WBC</td>
<td>1524</td>
<td>812</td>
<td>0.20</td>
</tr>
</tbody>
</table>

$^1$ the calculated free fraction in plasma was used
Figure 1a:

![Graph showing concentration (ng/ml) over time (h) for different tissues and WBC on day 1.](image)

Figure 1b:

![Graph showing concentration (ng/ml) over time (h) for different tissues and WBC on day 3.](image)
Figure 2a:

![Graph showing concentration of different tissue types over time on day 1.](image)

Figure 2b:

![Graph showing concentration of different tissue types over time on day 3.](image)
Figure 3: Ratios of AUC parent compound/metabolite over days for plasma, muscle, subcutis, and WBC.