RESEARCH ARTICLE

Pharmacokinetics of the prodrug thiamphenicol glycinate and its active parent compound thiamphenicol in beagle dogs following intravenous administration

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Abstract
1. This study investigated the pharmacokinetics of thiamphenicol glycinate (TG) and thiamphenicol (TAP) in beagles (n = 6) after intravenous administration of 50 mg/kg TG hydrochloride. Plasma concentrations of TG and TAP were measured by a HPLC-UV method.
2. Two-compartment model was selected to describe the pharmacokinetic characteristics of TG and TAP in vivo. Main parameters were as follows: AUC0–∞ of TAP and TG were 16,328 ± 1682 µg·min/mL and 3943 ± 546 µg·min/mL, respectively. The total plasma clearance (CL) of TG and TAP were 12.7 ± 2.0 mL/min/kg and 2.5 ± 0.3 mL/min/kg, respectively. Mean residence time (MRT) of TG and TAP were 27.5 ± 3.5 and 207.2 ± 20.2 min, respectively. The transformative rate constant (k10) from TG to TAP was 0.0477 ± 0.0028 min−1. The elimination rate constant (kM10) from TAP was 0.0238 ± 0.0044 min−1. Coefficients of variation (CV) between observed values and predicted ones were 5.9% and 18.2%, respectively. The volume of distribution of the central compartment for TG (Vc) and TAP (VCM) were 0.264 ± 0.022 L/kg and 0.127 ± 0.023 L/kg, respectively.
3. Pharmacokinetic parameters suggested that TG was presumably cleaved quickly by tissue esterase to release TAP for effectiveness in beagles after administration.

Keywords: Pharmacokinetics, thiamphenicol glycinate, thiamphenicol, beagle dog, prodrug

Introduction
Chloramphenicol, a potent bacteriostatic agent, is restricted to serious infections when other drugs are not effective or more toxic because of its lethal complication of aplastic anaemia (Turton et al., 2000). Its analogue, thiamphenicol (TAP) [(+)threo-2-di-chloroacetamido-1-(4-methylsulphonylphenyl)propane-1,3-diol] (Figure 1), is developed by replacing the aromatic nitro group with a methylsulphonyl group to limit the toxicity (Kitamura et al., 1997; Drago et al., 2000). Its broad antibiotic spectrum includes both Gram-negative and Gram-positive bacteria involved in upper and lower respiratory tract infections, bacterial prostatitis, and sexually transmitted diseases evoked by most Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Moraxella catharralis, and Haemophilus influenzae, as well as anaerobes (Tullio et al., 2004). It is generally recognized that TAP has an essential bacteriostatic activity by binding to the 50S subunits of the 70S ribosomes to block peptidyl transferase, hence inhibiting the extension of peptide chain and synthesis of bacterial protein. So TAP, in many countries and regions, has been widely used for therapeutic purposes not only in clinical practice but

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also in veterinary medicine (Turton et al., 2000, 2002). Furthermore, unlike chloramphenicol, the use of TAP has not been associated with any documented case of aplastic anaemia or grey syndrome (Li et al., 2002).

TAP, however, has been limited to oral administration because of its low water solubility. In order to develop parenteral formulation with improved water solubility, thiamphenicol glycinate (TG) (Figure 1), the ester prodrug of TAP, has been synthesized by esterifying the primary hydroxyl group with glycine. Unlike TAP, TG has high water solubility and can be readily developed for convenient parenteral formulation. TG is presumably cleaved by tissue esterase in vivo (Drago et al., 2000). Although many studies have been carried out in animals receiving TAP (such as cattle, sheep, and sea bass), few reports could be found for its prodrug TG in dogs (Abdennebi et al., 1994a, b; Intorre et al., 2002). In this study, pharmacokinetic parameters of TG and TAP in beagle dogs were investigated to better understand the metabolism and disposition of TG and TAP in dogs. In addition to the pharmacokinetic analysis, a compartmental model was developed to fit the concentration-time profiles for both TG and TAP simultaneously.

Materials and methods

Animals

Healthy adult beagle dogs of both sexes aged 20–24 months (7.5–8.8 kg) were purchased from the Experimental Animal Center of Southeast University (animal certificate number: SCXK 2002-0006). Animals were housed under controlled conditions (temperature: 20 ± 2°C, relative humidity: 50 ± 20%) with a natural light–dark cycle and were allowed to adapt to the housing environment for 1 week prior to the study. They were fasted 12 h before the experiment with free access to water. The present study was approved by the Animal Ethics Committee of China Pharmaceutical University.

Chemical reagents

The reference substances of TG, TAP, and TG hydrochloride were kindly offered by Henan Zhongshuai Medicine Science and Technology Development Limited Company (Zhengzhou, China). N-Acetyl-β-aminophenol (acetaminophen) was purchased from Jingshan Pharmaceutical Factory (Shantou, China) and was used as the internal standard (IS). HPLC-grade acetonitrile was the product of TEDIA Company (Fairfield, OH, USA). All other reagents were of analytical grade.

Preparation of stock solutions

Stock solutions of TG, TAP, and N-acetyl-β-aminophenol were prepared by, respectively, dissolving the accurately weighed compounds in 1 mL ethyl alcohol and diluted with water to reach final concentrations of 10 mg/mL for each compound. All stock solutions were stored at 4°C. Working solutions of TG, TAP, and N-acetyl-β-aminophenol were prepared by diluting each of the stock solutions to 1 or 2 mg/mL before use.

Drug administration and sample collection

Six beagle dogs (three males and three females) were used in this study. A single dose of 50 mg/kg TG hydrochloride (dissolved in 1.0 mL physiological saline) was intravenously administered at forearm vein to fasted beagle dogs by a bolus injection. Approximately 3 mL blood samples were collected from contralateral forearm vein into tubes (treated by heparin sodium) at 0, 5, 10, 20, 30, 45, 60, 90 min, and 2, 3, 4, 5, 6, 8, 10 h after administration. The blood samples were centrifuged at 800 g (about 4000 rpm) for 2 min and the plasma was stored at −70°C until analysis.

Sample preparation

To 500 µL plasma sample, 25 µL of 100 µg/mL IS working solution (dissolved in mobile phase) was spiked and briefly mixed before adding 2 mL ethyl acetate for extraction. After vortexed for 2 min, the samples were centrifuged at 800 g for 1 min and 750 µL of supernatant liquid was evaporated under a negative-pressure nitrogen stream by a rotated vaporizer. The residue was reconstituted in 250 µL mobile phase and 20 µL aliquot was injected manually into the HPLC system for analysis.

Assay methods for TG and TAP

The simultaneous determination of TG and TAP in beagle dogs’ plasma was conducted using a HPLC-UV method. The HPLC system consisted of a Model LC-10ATvp solvent delivery system (Shimadzu, Japan) and a SPD-10Avp UV detector (Shimadzu, Japan). The chromatographic separation was performed at ambient temperature using a Hypersil ODS 2 analytical column (5 µm, 4.6 mm × 200 mm, Elite Analytical Instruments Corporation, Dalian, China). The isocratic mobile phase was a mixture of acetonitrile–water (87:13, v/v) containing 0.003 M tetrabutyl ammonium bromide and 0.056 M ammonium acetate at a flow rate of 1 mL/min and the signal was monitored at wavelength of 224 nm.
Pharmacokinetic analysis

The maximal drug concentration ($C_{\text{max}}$) and the time of peak concentration ($T_{\text{max}}$) were derived directly from the experimental data. The total plasma clearance (CL), area under the curve (AUC), the first moment of the plasma concentration–time curve (AUMC), and the mean residence time (MRT) were estimated by applying a noncompartmental analysis to each individual time–concentration profiles of TG and TAP, respectively. For estimation of AUC$_{0-t}$ ($t = 180$ min for TG and $600$ min for TAP) and AUC$_{0-\infty}$, the linear trapezoidal estimation was used for the initial ascending portion of the curve and the logarithmic trapezoidal estimation was used for the descending portion of the curve. AUC$_{0-\infty}$ was calculated by extrapolation of AUC$_{0-t}$ to infinity by $C(t)/k_{1\text{M}}$ or $C(t)/k_{3\text{M}}$. The CL was obtained as dose/AUC$_{0-\infty}$ and the MRT was calculated as AUMC$_{0-\infty}$/AUC$_{0-\infty}$.

For the in-depth understanding of the kinetic conversion from TG to TAP, the compartmental analysis by simultaneously fitting time–concentration profiles of TG and TAP was necessary. Various user-defined pharmacokinetic models using the WinNonlin computer program (Version 4.0; Pharsight Corp., Mountain View, CA) were attempted. According to the principles for the model discrimination (Francis, 1997), the mechanistic pharmacokinetic models for TG and TAP were finally developed on the basis of two-compartment model for each as described by the following differential equations:

$$
\frac{dC_1}{dt} = k_{12} \cdot C_2 - (k_{12} + k_{1\text{M}}) \cdot C_1
$$

$$
\frac{dC_2}{dt} = k_{21} \cdot C_1 - k_{21} \cdot C_2
$$

$$
\frac{dC_{\text{M1}}}{dt} = k_{3\text{M}} \cdot C_1 \cdot V_c/V_{\text{CM}} - k_{3\text{M1}} \cdot C_{\text{M1}} - k_{2\text{M1}} \cdot C_{\text{M1}} - k_{1\text{M2}} \cdot C_{\text{M2}}
$$

$$
\frac{dC_{\text{M2}}}{dt} = k_{2\text{M1}} \cdot C_{\text{M1}} - k_{2\text{M2}} \cdot C_{\text{M2}}
$$

where $V_c$ and $V_{\text{CM}}$ were the volume of distribution in the central compartment for TG and TAP, respectively. $C_1$ and $C_2$ were concentrations of TG in central and peripheral compartments, and $C_{\text{M1}}$ and $C_{\text{M2}}$ represented concentrations of TAP in central and peripheral compartments, respectively. The drug concentrations of either TG or TAP at each time point were pooled for the compartmental analysis. Due to the large range of the drug concentrations, we assumed a constant relative error model, that is, the fitting was weighted by means of $1/C^2_\text{predicted}$. The pharmacokinetic model of TG and TAP in dogs was shown in Figure 2. The abbreviation i.v. represented intravenous administration. The $k_{12}$ and $k_{3\text{M2}}$ represented a rate constant distributed from central compartment to peripheral one of TG and TAP, respectively. $k_{31}$ and $k_{2\text{M2}}$, represented a rate constant distributed from peripheral compartment to central one of TG and TAP, respectively. The $k_{1\text{M}}$ represented a rate constant transformed from central compartment of TG to that of TAP and the $k_{3\text{M1}}$ represented a rate constant eliminated from central compartment of TAP.

Statistical analysis

The experimental data were expressed as the means and standard deviation (SD) for the pharmacokinetic parameters obtained by the noncompartmental analysis. Due to the pooling approach for the compartmental analysis, the estimation of pharmacokinetic parameters and the coefficient of variation (CV) obtained by the WinNonlin computer program were presented.

Results

Method validation

For each analysis, nine-point calibration curves for TG and TAP were constructed with linear concentration range of 0.015–100 µg/mL and their low calibration ones ranging from 0.015 to 0.80 µg/mL were taken into consideration. Assay validation for TG and TAP indicated a limit of detection (LOD) of 0.010 µg/mL and limit of quantification (LOQ) of 0.020 µg/mL. LOD was calculated to be 3 on the basis of signal-to-noise ratio spiking at low concentrations in the plasma samples. LOQ was calculated to be 10 of signal-to-noise ratio. Three concentrations of the TG and TAP samples ($n=5$ for each concentration) were kept at $-20°C$ for 1 week to evaluate the frozen stability. Frozen stability was $97.6 \pm 3.6\%$, $93.3 \pm 3.1\%$, and $101.1 \pm 1.3\%$ for samples of low, medium, and high concentrations, respectively.

The retention time ($T_d$) of analytes was kept consistent over a 2-week period at $4.17 \pm 0.03$ min, $5.81 \pm 0.06$ min, and $11.4 \pm 0.3$ min for the IS, TG, and TAP ($n=5$ analyses), respectively. The extraction recovery rate at low, medium, and high concentrations were $99.1 \pm 3.7\%$, $101.1 \pm 2.2\%$, and $100.9 \pm 6.8\%$ for TG and $104.2 \pm 1.9\%$, $100.7 \pm 2.3\%$, and $99.9 \pm 1.7\%$ for TAP ($n=5$ analyses). The accuracies at low, media, and high concentrations of both TG and TAP (the values were 100 times of the ratio of the difference between the determined value and the true value to the true value) were: $5.2\%$, $5.8\%$, and $10.1\%$ for TG and $4.5\%$, $2.9\%$, and $3.6\%$ for TAP, respectively ($n=5$ measurements). The within-day CV, evaluated by analyzing samples of
three concentrations in the same day (n=5 for each concentration), was below 3.0% for low, 6.0% for medium, and 5.0% for high drug concentrations. The between-day CV determined by the analysis of samples with same concentrations on three consecutive days was below 10.0% for all samples. All these results complied with the criteria of FDA bioanalytical method validation (US FDA, 2001).

Noncompartmental analysis

The plasma concentration–time profiles of TG and TAP in dogs (n=6) after i.v. bolus administration of 50 mg/kg TG hydrochloride are shown in Figure 3. For all the concentration–time profiles in the study, the percentage of extrapolation to infinity was <10%, indicating that reliable AUC0–∞ could be obtained for the parameter estimation (i.e. low CV) as shown in Table 2.

Based on the noncompartmental analysis, two reasonable assumptions about the in vivo transformation from TG to TAP were made: (1) 100% transformation from TG to TAP in central compartment and (2) all metabolisms of TG and TAP occur in the central compartment. The proposed mechanistic two-compartment open model for both TG and TAP fit the concentration–time profiles of TG and TAP well as shown in Figure 4. The validity of the fitting was assessed by low correlation among parameters, no patterns in residual plots, as well as high precision for the parameter estimation (i.e. low CV) as shown in Table 2.

Table 1. Estimated pharmacokinetic parameters of thiamphenicol glycinate (TG) and thiamphenicol (TAP) in beagle dogs by noncompartmental analysis.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>TG</th>
<th>TAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{\text{max}}) (µg/mL)</td>
<td>147.7 ± 26.7</td>
<td>87.3 ± 22.5</td>
</tr>
<tr>
<td>(T_{\text{max}}) (min)</td>
<td>5.0 ± 0.0</td>
<td>21.3 ± 16.5</td>
</tr>
<tr>
<td>CL (mL/min/kg)</td>
<td>12.7 ± 2.0</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>AUC_{c1} (µg-min/mL)</td>
<td>3874 ± 567</td>
<td>15,401 ± 1322</td>
</tr>
<tr>
<td>AUC_{c2} (µg-min/mL)</td>
<td>3943 ± 546</td>
<td>16,328 ± 1682</td>
</tr>
<tr>
<td>AUMC_{c1} (µg-min²/mL)</td>
<td>93,102 ± 18,547</td>
<td>2653,572 ± 347,857</td>
</tr>
<tr>
<td>AUMC_{c2} (µg-min²/mL)</td>
<td>108,569 ± 21,647</td>
<td>3406,109 ± 666,811</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>27.5 ± 3.5</td>
<td>207.2 ± 20.2</td>
</tr>
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Table 2. Estimated pharmacokinetic parameters of thiamphenicol glycinate (TG) and thiamphenicol (TAP) in beagle dogs by compartmental analysis.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Estimate</th>
<th>CV (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{\text{in}}) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.00636</td>
<td>15.0</td>
</tr>
<tr>
<td>(k_{\text{12}}) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.0114</td>
<td>28.6</td>
</tr>
<tr>
<td>(k_{\text{30}}) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.0477</td>
<td>5.9</td>
</tr>
<tr>
<td>(V_{\text{c}}) (L/kg)</td>
<td>0.264</td>
<td>8.3</td>
</tr>
<tr>
<td>(k_{\text{10}}) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.114</td>
<td>34.6</td>
</tr>
<tr>
<td>(k_{\text{10}}) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.0354</td>
<td>18.3</td>
</tr>
<tr>
<td>(k_{\text{10}}) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.0238</td>
<td>18.2</td>
</tr>
<tr>
<td>(V_{\text{d1}}) (L/kg)</td>
<td>0.127</td>
<td>18.0</td>
</tr>
<tr>
<td>(\alpha) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.0558</td>
<td>7.3</td>
</tr>
<tr>
<td>(\beta) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.00988</td>
<td>27.0</td>
</tr>
<tr>
<td>(T_{\text{1/2,0}}) (min)</td>
<td>12.4</td>
<td>7.3</td>
</tr>
<tr>
<td>(T_{\text{1/2,2}}) (min)</td>
<td>70.2</td>
<td>27.0</td>
</tr>
<tr>
<td>(\alpha) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.166</td>
<td>28.7</td>
</tr>
<tr>
<td>(\beta) (min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.00505</td>
<td>5.0</td>
</tr>
<tr>
<td>(T_{\text{1/2,30}}) (min)</td>
<td>4.18</td>
<td>28.7</td>
</tr>
<tr>
<td>(T_{\text{1/2,50}}) (min)</td>
<td>137.4</td>
<td>5.0</td>
</tr>
<tr>
<td>(V_{\text{sh1}}) (L/kg)</td>
<td>0.145</td>
<td>25.7</td>
</tr>
<tr>
<td>(V_{\text{ps1}}) (L/kg)</td>
<td>0.407</td>
<td>7.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Coefficient of variation of the estimate, not reflective of inter-animal variability.

<sup>b</sup>Macro-constant estimated for TG and TAP as the secondary parameters.

\(V_{\text{sh}}\) and \(V_{\text{ps}}\) represent the peripheral distribution volume of TG and TAP, respectively.
**Discussion**

In our present work, extraction with ethyl acetate was essential to remove endogenous impurities in plasma samples. The concentrations of the drugs in the plasma samples were simultaneously analyzed by means of the HPLC-UV method, which measured the total concentration of TG and TAP including the extent of plasma protein binding of them in our study. One should expect, however, that it is rather low, and it has been reported to be low in many species (e.g. about 10% in camels and sheep) (Papich and Riviere, 2001; Al-Nazawi, 2005). Furthermore, LOQ of 0.020 µg/mL could fully meet the requirements of detection.

The plasma concentration of TG declined biexponentially from 147.7 ± 26.7 µg/mL at 5 min to 1.22 ± 0.35 µg/mL at 180 min. The plasma concentration of TAP increased initially because of transformation from TG and then declined from 87.3 ± 22.5 µg/mL with $T_{\text{max}}$ of 21.3 ± 16.5 min to 4.94 ± 2.18 µg/mL at 600 min. TG was not detected in the urine of rats following intravenous administration (unpublished observations). Combining the huge CL (12.7 ± 2.0 mL/min/kg) and the short MRT of TG (27.5 ± 3.5 min) with the fact that no detectable TG in urine, it was highly likely that it was rapidly and completely transformed from TG to TAP by tissue esterase with the proposed mechanism, which allowed key assumptions to be made in the following compartmental analysis.

When estimated by using the compartmental models, the transformative rate constant from TG to TAP, $k_{\text{IM}}$ (0.0477 ± 0.0028 min$^{-1}$), was larger than the eliminative rate constant of TAP, that is $k_{\text{MIO}}$ (0.0238 ± 0.0044 min$^{-1}$), which made the time-concentration profile of TAP equivalent to an oral administration where $K_{\text{e}} (k_{\text{IM}})$ was larger than $K_{\text{e}} (k_{\text{MIO}})$. And for both the prodrug and the parent compound, the half-life of the distribution phase ($T_{1/2D}$) was shorter than the elimination phase ($T_{1/2P}$).

Interestingly, the results suggested that TAP had high fraction distributed into the peripheral compartment compared with TG, which may be attributed to lower hydrophilic property of the TAP.

Apparent volume of distribution (V) is an important indication of the diffusion of the drug in tissues. In the present study, $V_{c}$ (0.264 ± 0.022 L/kg) was larger than $V_{p}$ (0.145 L/kg), whereas $V_{ch}$ (0.127 ± 0.023 L/kg) was only one-third of $V_{pm}$ (0.407 L/kg). These results suggest that TAP is a lipophilic drug and the relatively larger $V_{pm}$ may be related to physiochemical characteristics of drug.

Prodrugs are usually designed to improve absorption, distribution, metabolism, and excretion (ADME) properties or pharmacological selectivity of some drugs. The activity of tissue esterase has been widely used to cleave the ester bond of the prodrug to release the active compound. For example, enalapril is converted by esterase to the active enalaprilat (Phensri and Chotima, 2005), and valaciclovir is converted by esterase to the active aciclovir (Weller et al., 1993). TG, the prodrug of TAP, was cleaved quickly and completely by the tissue esterase to TAP for antimicrobial activity. Obviously, the pharmacokinetic characters of both the active form and the prodrug were desired for in-depth understanding of the in vivo pharmacodynamics. As the prodrug of TAP, TG is actually a new form of medication with the improved water solubility for intravenous administration. It is necessarily required by the regulation to understand the rate and extent of its elimination. This article reported the pharmacokinetic properties of both TAP and its prodrug TG for the first time in beagle dogs. The noncompartmental and compartmental pharmacokinetic analysis suggested that it was rapidly and completely transformed from TG to TAP in vivo.

**Conclusions**

In conclusion, pharmacokinetics of the prodrug TG and its active parent compound TAP in beagle dogs following the intravenous administration were characterized as a rapid and sustained transformation from TG to TAP, slow elimination, and possibly complete bioavailability. In addition, a user-defined mechanistic model was finally developed to best fit TG and TAP simultaneously, which could be applied to other drugs similar to them in the future.

**Declaration of interest**

None to declare.

**References**


