Pharmacokinetics and metabolism of pefloxacin in turkeys

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SUMMARY

The pharmacokinetics of pefloxacin (PFL) and its active metabolite norfloxacin (NFL) were investigated in turkeys after single intravenous (i.v) and oral (p.o) administrations of pefloxacin mesylate at a dose of 10 mg kg⁻¹. Compartmental and non-compartmental analyses were used. Following p.o. administration, PFL was absorbed slowly with time to reach Cmax at 5.59 h vs 5.17 h (compartmental vs non-compartmental analyses). The Cmax were 3.28 μg mL⁻¹ vs 5.81 μg mL⁻¹. Oral bioavailability values for PFL, evaluated according to pharmacokinetic approaches were 75.08% and 78.95%, respectively. After i.v. and p.o. administrations, the metabolite ratio (MR), defined as the ratio between the respective values of the area under the plasma concentration time curve of NFL and PFL, were 4.75% vs 2.71% for iv administration and 5.89% vs 7.04% for p.o. administrations.

Keywords: Pharmacokinetics, pefloxacin, norfloxacin, metabolite, turkey.

Introduction

Pefloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7(4-methyl-1-piperazinyl) quinolono-3 carboxylic acid] (PFL) belongs to a fluoroquinolone class of antibacterial agents. It has a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative microorganisms. The minimum inhibitory concentrations of PFL and its active metabolite norfloxacin (NFL) against bacterial pathogens like Escherichia spp., Klebsiella spp., Salmonella spp. and Haemophilus spp. ranged from 0.06 to 0.3 μg mL⁻¹ [5, 19].

PFL is partially metabolized in the liver to norfloxacin, pefloxacin N-oxide, pefloxacin glucuronide, oxepofloxacin, oxonorfloxacin [8]. NFL is a potent antimicrobial agent used in human and veterinary practice [17, 18]. As the other fluoroquinolones, PFL inhibits DNA gyrase enzyme and is intensely bactericidal against susceptible bacteria [14].

The clinical efficacy of an antimicrobial is determined not only by its ability to reach site of infections and its persistence within tissues. Pharmacokinetic variables such as plasma concentration, half-life, bioavailability, rate of elimination are important considerations for rational use of antimicrobial agents.

Kinetic evaluation of PFL has been carried out in humans, monkeys, cows, sheep, goats, calves, chickens, pigeons, ducks, rabbits, dogs, rats and mice, but there is a lack of information about the pharmacokinetics of PFL in blood serum of turkeys [1-4, 6, 7, 9, 10, 13, 15, 16, 20, 21].

The aim of this study was therefore to investigate the serum pharmacokinetics of PFL and its active metabolite NFL in turkeys following intravenous and oral administration.

Material and Methods

EXPERIMENTAL ANIMALS

The study was performed in 12 (6 male and 6 female) broiler turkeys (stock hybrid BUT-9, France) at the age of 7-8 months, weighing 5.8-8.2 kg body weight. The birds were provided by the Animal Breeding Institute - Kostinbrod, Bulgaria. The turkeys were under uniform conditions of housing and feeding, according to the species, requirements two weeks prior to the experiment and until the end of the study. The birds received a standard diet (not coccidiostatic or antibiotics). Water and feed were available ad libitum.

The turkeys were housed in metal cages in groups of 6, under conditions identical to those in the turkey-breeding farm of the Stara Zagora Breeding Base of the Animal Breeding Institute (Kostinbrod).

DRUGS

Pefloxacin mesylate was used as a powder (Chemos GmbH, Germany), dissolved ex tempore in distilled water for 1% oral
administration, and as 5% injectable solution for i.v. injection, after dilution with saline.

EXPERIMENTAL DESIGN

A two-way crossover design was applied, with a washout period of 20 days between the treatments. PFL was administered i.v., and into the crop in a dose of 10 mg kg\(^{-1}\) body weight. For the determination of i.v. pharmacokinetics the tested fluoroquinolone was bolus injected (short-term infusion) in the left v. brachialis. The p.o. administration of PFL was by instillation of the aqueous solution into the crop, using a semi-rigid tube, after 16 h of food deprivation.

Blood samples (1.0 mL) were collected prior to drug application (t = 0), and then at 0.17, 0.33, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after treatment, from the right brachial vein. The sera were separated via centrifugation (1800 x g for 10 min) and stored at -25°C until analyzed.

DRUG ANALYSIS

The concentrations of PFL and its metabolite NFL in blood serum were determined simultaneously by using reverse-phase HPLC with UV detection, according to the method described by MONTAY et al. [8] after sample extraction. In brief, 0.75 mL acetonitrile was added to 0.5 mL serum in a test tube. After vortex mixing at high speed for 15 sec, the tube was centrifuged (950 x g for 10 min). The clear supernatant was collected and twice the volume of HPLC grade water was added. The aliquot was then filtered through a 0.22-μm cellulose acetate membrane filter and 20 μL of filtrate was injected into the HPLC system. The HPLC (Shimadzu Europa GmbH, Duisburg, Germany) consisted of a double pump, manual loop injector and UV detector (SPD 10A) with software chromatopak for data analysis. The conditions of HPLC were as follows: Column C18, 4 x 250 mm (Shimadzu Europa GmbH, Duisburg, Germany), Mobile phase: acetonitrile: methanol: HPLC water (17:3:80, v/v/v) containing 0.4% triethylamine and 0.4% orthophosphoric acid (85%, v/v). The pH of the mobile phase was 2.5. The eluent was monitored at the wavelength of 278 nm with a flow rate of 0.6 mL min\(^{-1}\) at ambient temperature (20 ± 2°C). PFL and NFL were quantified from their respective peak areas and the concentrations in serum samples were determined (Figure 1).

The mean serum concentrations of PFL obtained after i.v. injection and serum disposition of PFL and its metabolite NFL following p.o. administration of pefloxacin mesylate were determined (Figure 1). The curve of the mean serum concentrations after i.v. PFL administration to turkeys indicated an initial rapid phase, corresponding to the distribution (α) of the drug with a half-life of 0.126 ± 0.02 h. The phase of the PFL distribution was followed by a slower elimination (β) phase with an elimination

PHARMACOKINETIC ANALYSIS

The pharmacokinetic parameters of PFL and its active metabolite NFL, after single i.v. and p.o. administration were calculated using WinNonlin computer programme, version 4.0.1 (Pharsight Corporation, Mountain View, CA, USA). The best fit was determined according to the Akaikes Information Criterion (AIC) [22]. The pharmacokinetic parameters of PFL and NFL were presented as mean ± standard deviation.

Compartmental and non-compartmental pharmacokinetic analyses were used to describe the pharmacokinetics of parent and metabolite compounds following single bolus injection or p.o. administration. Serum concentrations vs. time data best fitted to a two-compartment open model after i.v. injection of PFL and a one-compartment open model following p.o. dosing.

The following parameters were determined: the elimination half-life (t\(_{1/2}\)); half-life of distribution (t\(_{1/2α}\)); absorption half-life (t\(_{1/2abs}\)), half-life of metabolite formation (t\(_{1/2kf}\)), maximum concentration time (MAT), area under the serum concentration-time curve (AUC), total body clearance (Cl\(_B\)), volume of distribution at steady state (V\(_S\)), total body clearance (Cl\(_B\)), maximum serum concentration (C\(_{max}\)), time needed to reach peak serum concentration (T\(_{max}\)), metabolite ratio (MR) calculated as MR = (AUC\(_{NFL}\)/AUC\(_{PFL}\) x D\(_{p.o}\) x t\(_{1/2\text{p.o.}}\)) x 100. For the oral route of administration, the bioavailability (F) was determined, using the following equation: F (%) = (AUC\(_{PFL}\)/AUC\(_{i.v}\)) x D\(_{i.v}\) x t\(_{1/2\text{i.v.}}\) /AUC\(_{i.v}\) x D\(_{p.o}\) x t\(_{1/2\text{p.o.}}\) x 100.

Results

The mean serum concentrations of PFL obtained after i.v. injection and serum disposition of PFL and its metabolite NFL following p.o. administration of pefloxacin mesylate were determined (Figure 1).

FIGURE 1: Serum concentrations (mean ± SD) of PFL and its active metabolite NFL after single i.v. and p.o. administration in a dose 10 mg kg\(^{-1}\) body weight.
The half-life of PFL was 3.42 ± 0.39 h and 2.44 ± 0.21 h, according to the respective compartmental vs non-compartmental analysis method.

After p.o. administration of the drug, an appreciable concentration of PFL (0.101 ± 0.048 µg mL⁻¹) appeared in serum at 0.17 h, and the mean peak concentration (5.81 ± 1.17 µg mL⁻¹) was achieved at 5.17 h. The serum NFL concentration at 4 h was 0.413 ± 0.011 µg mL⁻¹ which declined to 0.016 ± 0.004 µg mL⁻¹ at 12 h. PFL serum concentrations remained detectable (≥ 0.10 µg mL⁻¹) up to 12 h.

Mean pharmacokinetic parameters of PFL and its metabolite after p.o. administration of pefloxacin mesylate are presented in Table I and Table II.

The drug-concentration vs. time curves after p.o. PFL administration best fitted to a one-compartment open model.

Discussion

In the present study, the pharmacokinetics of PFL and its active metabolite NFL were determined in healthy male and female turkeys after i.v. and p.o. administration of pefloxacin mesylate at a dose of 10 mg kg⁻¹ body weight. The disappearance of PFL from the blood serum of turkeys was characterized by an initial rapid distribution phase followed by a slower elimination phase. PFL has a shorter t₁/₂ in turkeys

### Table I: Selected pharmacokinetic parameters (mean ± SD) of PFL and its metabolite NFL after single i.v. injection of pefloxacin mesylate at a dose 10 mg kg⁻¹ body weight

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Pefloxacin Compartmental analysis</th>
<th>Pefloxacin Non-compartmental analysis</th>
<th>Norfloxacin Compartmental analysis</th>
<th>Norfloxacin Non-compartmental analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₁/₂α</td>
<td>h</td>
<td>0.126 ± 0.02</td>
<td>-</td>
<td>0.179 ± 0.02</td>
<td>-</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>h</td>
<td>2.44 ± 0.21</td>
<td>3.42 ± 0.39</td>
<td>3.36 ± 0.72</td>
<td>5.70 ± 0.37</td>
</tr>
<tr>
<td>t₁/₂kf</td>
<td>h</td>
<td>-</td>
<td>-</td>
<td>3.61 ± 0.54</td>
<td>-</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>3.01 ± 0.30</td>
<td>3.78 ± 0.22</td>
<td>4.98 ± 0.30</td>
<td>4.07 ± 0.48</td>
</tr>
<tr>
<td>Vss</td>
<td>L kg⁻¹</td>
<td>0.742 ± 0.09</td>
<td>0.823 ± 0.08</td>
<td>35.410 ± 0.11</td>
<td>31.810 ± 0.31</td>
</tr>
<tr>
<td>ClB</td>
<td>ml min⁻¹ kg⁻¹</td>
<td>4.16 ± 0.21</td>
<td>3.61 ± 0.24</td>
<td>125.00 ± 1.20</td>
<td>130.00 ± 0.86</td>
</tr>
<tr>
<td>AUC₀→LOQ</td>
<td>µg h mL⁻¹</td>
<td>-</td>
<td>46.58 ± 3.23</td>
<td>-</td>
<td>1.220 ± 0.40</td>
</tr>
<tr>
<td>AUC₀→∞</td>
<td>µg h mL⁻¹</td>
<td>40.65 ± 2.26</td>
<td>47.23 ± 3.28</td>
<td>1.93 ± 0.40</td>
<td>1.280 ± 0.63</td>
</tr>
<tr>
<td>MR</td>
<td>%</td>
<td>-</td>
<td>4.75</td>
<td>-</td>
<td>2.71</td>
</tr>
</tbody>
</table>

### Table II: Pharmacokinetic parameters (mean ± SD) of PFL and its metabolite NFL following single p.o. administration of pefloxacin mesylate at a dose 10 mg kg⁻¹ body weight

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Pefloxacin Compartmental analysis</th>
<th>Pefloxacin Non-compartmental analysis</th>
<th>Norfloxacin Compartmental analysis</th>
<th>Norfloxacin Non-compartmental analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₁/₂</td>
<td>h</td>
<td>3.64 ± 0.19</td>
<td>4.36 ± 0.25</td>
<td>6.01 ± 0.23</td>
<td>5.19 ± 0.52</td>
</tr>
<tr>
<td>t₁/₂abs.</td>
<td>h</td>
<td>2.14 ± 0.18</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>t₁/₂kf</td>
<td>h</td>
<td>-</td>
<td>-</td>
<td>4.65 ± 0.48</td>
<td>-</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>10.84 ± 0.53</td>
<td>8.21 ± 0.25</td>
<td>12.01 ± 0.79</td>
<td>8.54 ± 0.64</td>
</tr>
<tr>
<td>MAT</td>
<td>h</td>
<td>7.83 ± 0.27</td>
<td>4.43 ± 0.32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AUC₀→LOQ</td>
<td>µg h mL⁻¹</td>
<td>-</td>
<td>35.483 ± 4.42</td>
<td>-</td>
<td>2.48 ± 0.10</td>
</tr>
<tr>
<td>AUC₀→∞</td>
<td>µg h mL⁻¹</td>
<td>45.530 ± 4.89</td>
<td>36.784 ± 4.67</td>
<td>2.683 ± 0.76</td>
<td>2.59 ± 0.10</td>
</tr>
<tr>
<td>Cmax</td>
<td>µg mL⁻¹</td>
<td>3.280 ± 0.46</td>
<td>5.81 ± 1.17</td>
<td>0.228 ± 0.32</td>
<td>0.155 ± 0.01</td>
</tr>
<tr>
<td>Tₘax</td>
<td>h</td>
<td>5.59 ± 0.12</td>
<td>5.17 ± 0.54</td>
<td>4.67 ± 1.17</td>
<td>2.00 ± 0.55</td>
</tr>
<tr>
<td>F</td>
<td>%</td>
<td>75.08 ± 3.14</td>
<td>78.95 ± 1.43</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MR</td>
<td>%</td>
<td>-</td>
<td>5.89</td>
<td>-</td>
<td>7.04</td>
</tr>
</tbody>
</table>

**Legend:**

- t₁/₂ - half-life of elimination;
- t₁/₂α - half-life of absorption;
- t₁/₂kf - half-life of metabolite formation;
- MRT - mean residence time;
- Vss - steady-state volume of distribution;
- ClB - total body clearance;
- AUC₀→LOQ - area under the concentration-time curve from 0 to last detected concentration;
- AUC₀→∞ - area under the concentration-time curve from 0 to ∞;
- MR - metabolite ratio.

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after both routes of administration than in other avian species such as chickens and ducks [3, 6, 15], and larger than in pigeons [13]. The $t_{1/2}$ of parent substance (calculated by compartmental and non-compartmental analysis) was increased by 49% and 21%, respectively, after p.o. administration, compared with i.v. injection. $V_{ss}$ after both routes indicates that PFL easily penetrated all tissues, in agreement with data reported earlier for the tested gyrase inhibitor [6, 7, 10, 16, 20].

When given orally, PFL was slowly, but well absorbed from the digestive system of the turkeys. It has a larger $T_{max}$ in tested avian species than other birds and mammals - pigeons (1.16 h), chickens (3.33 h), ducks (1.35 h), sheep (1.44 h) and goats (2.30 h) [3, 7, 10, 11, 15].

Mean maximal serum concentrations of PFL following p.o. administration of PFL in this study were nearly the same as the one determined in broilers chickens (3.78 μg mL⁻¹) [15], but higher than that in pigeons (2.90 μg mL⁻¹) and ducks (1.35 μg mL⁻¹) [3, 11].

The broiler chickens, pigeons and ducks, similarly to the turkeys, were treated p.o. with pefloxacin mesylate at the same dose.

The obtained data for MAT and $t_{1/2abs}$ were evidences for the delayed process of absorption after p.o. application in turkeys, which is in contrast to pigeons (0.17 h), ducks (0.33 h), sheep (0.44 h) and goats (2.30 h) [3, 7, 10, 11, 15].

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We determined, that after p.o. administration to turkeys, the elimination half-life and MRT of parent substance (PFL) are shorter than those in ducks (4.97 h for male and 6.45 h for female birds), pigeons (6.68 h), and broiler chickens (8.54 h or 8.74 h), respectively [3, 6, 11, 15].

The hypothetical causes for these differences could be in the species and breed peculiarities of the treated birds.

The bioavailability, determined after p.o. application, is higher than that in ducks (60.31% - for male and 68.71% - for female birds), pigeons (73.42%), and goats (42.42%), but slightly smaller than in sheep (82.42%) [3, 7, 10, 11].

It is known, that after administration to humans and different animal species, PFL is metabolized to 5 metabolites - pefloxacin N-oxide, pefloxacin glucuronide, oxopefloxacin, N-dimethyl pefloxacin (norfloxacin) and oxonofloxacin, but only norfloxacin has antimicrobial activity [6, 9].

PFL metabolism is extensive mainly via oxidation to form the principal metabolite N-dimethyl pefloxacin (NFL). There is no difference between the activity of PFL (parent drug) and the active metabolite (NFL) against most Gram-negative species such as Enterobacteriaceae, Haemophilus, Neisseria, Legionella, Campylobacter [6].

In the present study, serum concentrations of the active metabolite NFL were increased very rapidly (at 0.17 h) after both routes of administration of parent substance. Their $C_{max}$ was reached at 4 h following p.o. administration of PFL to turkeys. It appears that time for reaching $C_{max}$ of NFL after p.o. treatment is identical to that of the parent substance. The elimination half-life and MRT of the metabolite NFL after both routes of application were longer than those of parent substance (Table I and Table II).

The results of the present study suggests that after i.v. or p.o. application in turkeys, PFL is subject to a relatively low conversion via oxidation, the result of which is a lower percentage of obtained active metabolite NFL, compared to the parent substance (see MR in Table I and Table II). Similar data are reported by ISEA et al. [6] for chickens (MR = 5.2%), from DIMITROVA et al. [3] for ducks (MR = 2.90% by male and MR = 6.3% by female birds; MR = 1.45% by male and MR = 1.41% by female birds), whereas PANT et al. [15] found a higher metabolite ratio for NFL in chickens (22%) as compared to our results and these of ISEA et al. [6], and DIMITROVA et al. [3].

References

PHARMACOKINETICS OF PEFLOXACIN IN TURKEYS


