OCULAR PHARMACOKINETICS OF ENROFLOXACIN IN DOGS

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Received for publication February 08, 2007

Abstract

Eight dogs at 2 and 3 years of age and weighing 11-14 kg were used. The dogs were divided into two groups. The first group was administered with enrofloxacin by intra-vitreal route at a dose of 2 mg/eye, whereas the second group was administered the same antibiotic at the same dose by subconjunctival route. Subsequent to the administration, the samples of the aqueous humour were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, 24, and 72 h. Slope factor (α, β), ka (absorption rate constant), absorption half-life (t 1/2a), half-life of elimination in the aqueous humour (t 1/2β), mean residence time in the aqueous humour (MRT), area under the concentration time curve from zero up to ∞ (AUC 0→∞), maximal concentration in the aqueous humour after subconjunctival administration (C max), and time needed to reach C max (t max) values were assessed by means of pharmacokinetic analyses. Accordingly, a statistically significant difference was demonstrated between groups in t 1/2β, MRT, and AUC 0→∞ values. No statistically significant difference was observed between groups with respect to the other parameters. In conclusion, the administration of enrofloxacin by intra-vitreal route was considered to be a rapid and effective method for the treatment of intra-ocular infections than subconjunctival application. After intra-vitreal drug application, t 1/2β, MRT, and AUC 0→∞ were 9.47±3.79 h, 7.77±2.10 h, and 202.18±41.80 µg/h/mL, respectively, while after subconjunctival application these values were 3.84±1.30 h, 4.75±1.11 h, and 10.29±8.76 µg/h/mL.

Key words: dogs, enrofloxacin, ocular pharmacokinetics.

Enrofloxacin is a compound with the following chemical structure: 1-cyclopropyl-7-(4-ethyl-1-piperazyl)-6-fluoro-1, 4-dihydro-4-oxo-3 quinolone carboxylic acid. Differently than in other antibiotics, the quinolone nucleus of this compound possesses a CH3 (methyl) group bound to nitrogen in the 1st position in the A ring, and a C2H5 group bound to nitrogen in the 7th position in the B ring (2, 8, 9). Enrofloxacin selectively inhibits the activity of DNA-gyrase (14, 17, 20). It is a broad-spectrum antibiotic with bacteriocidal effect against the majority of aerobic and facultative anaerobic bacteria, Mycoplasma, and Rickettsia. The presence of a fluor group in the chemical structure of the compound increases the efficacy of the antibiotic against Gram-positive and aerobic bacteria (11, 12, 14, 19, 21). Plasmid-mediated resistance to enrofloxacin has not been reported. The main mechanism of the resistance to fluoroquinolones involves chromosomal changes. This results in enzymatic changes (DNA-gyrase) and subsequent decrease in passage of fluoroquinolones to bacteria (14). The quinolone class of antibiotics has been used for the treatment of eye infections over a long period. One of the most widely utilised compounds is ciprofloxacin that, in the form of eye drops, is suitable for the topical use (1, 3, 7, 16). Despite reports of related studies carried out in various animal species (11-13) and humans (16) with quinolone antibiotics, most of these studies have concerned the passage of antibiotics that have been administered by oral and parenteral routes into the eyes.

This study was aimed at determination of the possible subconjunctival and intra-vitreal administration of enrofloxacin, in the treatment of eye infections, particularly intra-ocular infections. It should be noted that so far enrofloxacin has not been yet formulated for the use in eye infections.

Material and Methods

Animals. Eight mixed breed male dogs at 2 and 3 years of age and weighing 11-14 kg were used. The dogs were divided into two groups. Enrofloxacin was administered by intra-vitreal (group I) or subconjunctival (group II) route in a 2 mg/eye dose. Samples of the aqueous humour (20 µl volume) were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, 24, and 72 h after antibiotic administration by a needle gently inserted into the anterior part of the eye. Drug application and sample collection were performed using the right eye of each animal in both groups. The samples were stored at -20 °C until analysed. Prior to the sample collection, local anaesthesia with 0.5% Alcaine® with neither adrenaline nor antibiotic was applied.
**Antibiotic analysis.** Extraction and preparation of the samples were carried out as described by Rizk et al. (15) with minor modifications. The spectrum of the samples read the calculation measured spectrofluorimetrically (Shimadzu RF 5301) using a programme called RFPc at 277 excitation and 240-600 nm wavelength. Using the same programme, 10 points were determined between 420-520 nm wavelengths by interval 10 nm. These absorbances were evaluated by comparing with standard absorbance points. A plot was obtained from standard absorbances and the absorbances from the samples were used to quantify antibiotic concentrations (µg/mL). The sensitivity of the method was 10 ng/mL and the recovery was 97.60%.

**Pharmacokinetic analysis.** A model that would be based on the distribution of the drug in pharmacokinetic calculations was accomplished by regression analysis, which was obtained using aqueous humour-time curve by taking r² values into account. Slope factor (α, β), kₐ (absorption rate constant), absorption half-life (t₁/₂a), half-life of elimination in the aqueous humour (t₁/₂β), mean residence time in the aqueous humour (MRT), area under the concentration time curve from zero up to ∞ (AUC₀→∞), maximal concentration in the aqueous humour after subconjunctival administration (Cmax), and time needed to reach Cmax (tmax) values were assessed by means of pharmacokinetic analyses by using a packet programme, PKCALC, which contained equations reported by Shumaker (18).

**Statistical analysis.** The SPSS package programme for Windows was utilised in statistical analyses. The data was given in the form of an arithmetical mean values and standard deviations. Comparison of the groups was performed by the means of the Mann-Whitney U test, and by using the SPSS for Windows 10.0 programme.

**Results**

The examination of the aqueous humour-time curve revealed a high antibiotic concentration after intra-vitreal application, but low after administration by the subconjunctival route. Alternatively, pharmacokinetic analyses demonstrated a statistically significant difference between groups with regard only to t₁/₂β, MRT and AUC₀→∞ values. No significant difference was determined between the groups with regard to the other parameters (Table 1). However, when aqueous humour-time curve was analysed, enrofloxacin was found to be within the detection limit until 24 h after intra-vitreal application; and 6 h after subconjunctival application (Fig. 1). The statistically significant difference between AUC₀→∞ values supports these findings.

The level of enrofloxacin in the aqueous humour, subsequent to administration by intra-vitreal route, was found to be 92.18 µg/mL, 50.56 µg/mL, 14.33 µg/mL, and 1.083 µg/mL at 0.083, 1, 4, and 24 h, respectively (Fig. 1). On the other hand, the level of subconjunctivally administered enrofloxacin in the aqueous humour was 2.52 µg/mL, 4.881 µg/mL, 1.597 µg/mL, and 0.836 µg/mL at 0.083, 0.5, 2, and 6 h, respectively.

![Fig. 1. Distribution of enrofloxacin in the aqueous humour after intra-vitreal (group 1) and subconjunctival (group 2) application.](image-url)

**Table 1**

Aqueous humour pharmacokinetic parameters of enrofloxacin for the 72 h after intra-vitreal and subconjunctival administration at a dose of 2 mg/eye

<table>
<thead>
<tr>
<th>Parameters**</th>
<th>Intra-vitreal application</th>
<th>Subconjunctival application</th>
</tr>
</thead>
<tbody>
<tr>
<td>α (h⁻¹)</td>
<td>1.00±0.57</td>
<td>0.46±0.16</td>
</tr>
<tr>
<td>β (h⁻¹)</td>
<td>0.13±0.10</td>
<td>0.19±0.06</td>
</tr>
<tr>
<td>kₐ (h⁻¹)</td>
<td>-</td>
<td>13.49±5.09</td>
</tr>
<tr>
<td>t₁/₂a (h)</td>
<td>0.89±0.54</td>
<td>1.15±0.80</td>
</tr>
<tr>
<td>t₁/₂β (h)</td>
<td>9.47±3.79</td>
<td>3.84±1.30*</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>7.77±2.10</td>
<td>4.75±1.11*</td>
</tr>
<tr>
<td>AUC₀→∞ (µg/h/mL)</td>
<td>202.18±41.80</td>
<td>10.29±8.76*</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>-</td>
<td>6.09±3.38</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>-</td>
<td>4.96±4.53</td>
</tr>
</tbody>
</table>

*P<0.05, **α, β: slope factor; kₐ: absorption rate constant at; t₁/₂a: distribution half-life in aqueous humour; t₁/₂β: half-life of elimination in aqueous humour; t₁/₂: absorption half-life; MRT: mean residence time in aqueous humour; AUC₀→∞: area under the concentration time curve from zero up to ∞; Cmax: maximal concentration in aqueous humour after subconjunctival administration; tmax: time needed to reach Cmax.
Discussion

Regression analysis of the administration by intra-vitreal route, demonstrated that the distribution of the antibiotic was in accordance with the two-compartment model. It has been reported that enrofloxacin given in different ways to various animals species shows similar distribution model (4-6). The examination of the level of the antibiotic revealed drug concentrations to be much higher after intra-vitreal administration, when compared to administration by a subconjunctival route.

Pharmacokinetic analyses demonstrated that the distribution half-life of the antibiotic was shorter after intra-vitreal administration in comparison to subconjunctival application. The excretion half-life was determined to be longer after subconjunctival administration. Changes of the determined to be longer after subconjunctival subconjunctival application. The excretion half-life was determined to be longer after administration by intra-vitreal and subconjunctival route. The mean period of maintenance of the antibiotic in the aqueous humour was determined to be longer after administration by intra-vitreal route. AUC0→∞ was found to be quite low at subconjunctival administration than after intra-vitreal administration. There was a statistically significant difference (P< 0.05) between both routes for AUC0→∞, MRT, and t1/2. This is also evident from the markedly lower antibiotic concentration in the aqueous humour when compared to the administration by intra-vitreal route. Therefore, in order to obtain a high concentration of antibiotic in the eye, either the drug should be administered at much higher doses in comparison to intra-vitreal administration, or the doses should be repeated at short intervals. Low absorption from the site of administration, and passage of a large part of the antibiotic into systemic blood circulation by the surrounding capillary veins, takes place amongst the underlying reasons for the low bioavailability of the antibiotic at subconjunctival administration. There are many studies that have been carried out on the ocular pharmacokinetics of quinolone antibiotics. In these studies, the passage of the antibiotic from the systemic blood circulation into the eye as well as the pharmacokinetic profile has been examined (11-13). In severe eye infections, it may be obligatory to apply the drug directly to the eye (10), in order to obtain the drug’s effectiveness in a short time, and to increase a better chance of treatment. A study carried out within this framework with quinolone antibiotics including enrofloxacin has not been come across. For this reason, pharmacokinetic data obtained from this study could not be compared with similar data. However, this study bears scientific significance with respect to being a reference for the future studies.

In conclusion, comparison of two routes of administration of enrofloxacin to the eye revealed that the treatment period after subconjunctival administration was shorter than that after the intra-vitreal administration. Therefore, the drugs administered by subconjunctival route should be given more frequently than those applied intra-vitreally.

References