PLASMA KINETICS AND TISSUE RESIDUES OF FENBENDAZOLE FOLLOWING ORAL ADMINISTRATION TO PIGS

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Twelve pigs received single oral dose of fenbendazole (5.0 mg/kg b.w.) and samples of blood from eight animals were collected at 0, 2, 4, 8, 12, 24, 36, 48, 96 and 120 h. Pigs were then slaughtered in groups of four on 4th, 6th and 8th d after administration of the drug and samples of the kidneys, liver and muscles were collected. Fenbendazole (FBZ) and its metabolites: fenbendazole sulfoxide (FBZ-SO) and fenbendazole sulfone (FBZ-SO₂) in all the collected samples were analysed by the use of high pressure liquid chromatography (HPLC) with UV detection.

The parent compound was present in plasma only in the samples collected 2 h after drug administration (mean 0.015 µg·ml⁻¹). Plasma concentrations of metabolites reached their maximum in the samples collected 8 h after drug administration (mean 0.116 ± 0.029 µg·ml⁻¹) and declined to the levels below the detection limit by 60 h. Peak plasma concentrations of metabolites (Cmax) was 0.133 µg·ml⁻¹. The total area under the plasma concentration versus time curve (AUC) was 3.5 µg·h/ml.

In samples of tissues (kidneys, liver and muscles) no parent drug was detected, whereas the minute amounts of metabolites (well below MRLs) were found in pig’s liver on day 4 following a single oral administration of the drug.

Key words: pigs, fenbendazole, pharmacokinetics, residues.

Fenbendazole (FBZ), a member of the benzimidazole group of anthelmintics is still highly effective against all stages of gastrointestinal nematodes in pigs, including inhibited larval stages and lungworms as reviewed by Ungemach (13) and Einstein et al. (4). Fenbendazole has been approved by the European Agency for the Evaluation of Medical Products (EMEA) for the use as an anthelmintic in swine. It is administered with feed in the dose of 3-5 mg/kg for 5-10 d, to control Ascaris, Trichuris, Oesophagostomum, Strongylides and Hyastrongylus spp.

After oral administration fenbendazole is absorbed from the intestine and extensively metabolized by microsomal oxidation in the liver. Important metabolites of FBZ are: fenbendazole sulfoxide (FBZ SO), which is also effective as an anthelmintic and fenbendazole sulfone (FBZ SO₂) (3).
The use of FBZ as an anthelmintic in food producing animals requires the withdrawal period to be established in relation to its maximum residue limit (MRL). In 1997 the Committee for Veterinary Medicinal Products (CVMP) of the EU fixed the MRLs for FBZ in edible tissues in all species as the sum of all residues that can be oxidized to FBZ-sulfone (parent drug, its sulfoxide and sulfone). The established MRLs in pork were 500 µg·kg⁻¹ in the liver and 50 µg·kg⁻¹ in muscle, kidney and fat (2).

In recent years several studies have been reported on the kinetics and disposition of FBZ and its metabolites in cattle, sheep and goats (7, 9, 11), but only a few data are available in the literature about kinetics and residues of this drug in swine (1, 10).

This is probably the reason of discrepancies between withdrawal periods established for different fenbendazole formulations for pigs (2 to 20 d), as described in previous paper (11).

The aim of the present studies was to investigate plasma kinetics of fenbendazole and its metabolites and elimination of residues from the tissues after a single oral administration of fenbendazole to swine.

Material and Methods

Animal experiment. Twelve clinically healthy pigs (six males and six females) weighing 35.5 – 47.5 kg were used in the study. The animals were kept in individual pens with free access to water and fed a proprietary pig feed in amounts adjusted to their body weight.

Fenbendazole (Fenbesan pulver 4%, Polfa, Kutno) was administered in a single oral dose of 5.0 mg/kg bw. The FBZ dose, calculated for each animal, was put into a small amount of feed and given to the animals in the morning. After it had been fully consumed, the regular daily ration was given.

Blood samples were collected from eight pigs by jugular vein puncture at 0, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96 and 120 h after drug administration. Plasma was separated and stored frozen at -17°C until analysed. The animals were slaughtered in groups of four at 4, 6 and 8 days after drug administration. Samples of the kidneys, liver and muscles were collected and stored frozen (-17°C) until they were analysed.

The experiment was carried out according to the EU guidelines for the testing of veterinary medicinal products (6). The EU principles of animal protection (Council Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes) as well as the national regulation on the protection of animals (Ustawa o ochronie zwierząt Dz.U. nr 111 z dnia 21.08.1997) were followed.

Analytical method. FBZ, FBZ sulfoxide and FBZ-sulfone were quantified in plasma by HPLC with UV detection, according to the method described previously (12). The lower limits of quantification of the method were 0.01 µg·ml⁻¹ of FBZ and 0.005 µg·ml⁻¹ of FBZ-sulfoxide and FBZ-sulfone. Indications of the detector were linear over a tested range of 0.005-0.2 µg·ml⁻¹. The linear correlation coefficient was 0.998 for FBZ and 0.999 for FBZ-sulfoxide and FBZ-sulfone. Precision of the method was 20.5% for the samples fortified on the level of 0.05 µg·ml⁻¹.

Pharmacokinetic and statistical analysis. Pharmacokinetic analysis of data was performed to fit individual animal data sets in a least square nonlinear regression analysis. Standard pharmacokinetic parameters were calculated according to noncompartmental pharmacokinetic model, using the computer program MK Model Ver. 5 (Biosoft, Cambridge 1994).

The area under the plasma concentration versus time curve (AUC) and area under the first moment curve (AUMC) were calculated according to the trapezoidal rule. Mean residence time (MRT) was calculated according to the equation $\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$. Peak plasma concentrations (Cmax) and times to reach Cmax (Tmax) for metabolites of fenbendazole were determined by visual inspection of the individual plasma concentrations versus time curves.

To establish withdrawal time for edible pig tissues, the confidence belts of the decay curves of total fenbendazole plasma levels were constructed applying linear regression analysis by Sigma Plot (Jandel Scientific GmBH, 1994). A withdrawal time was assigned on the base of the point of intersection of the MRL value with upper confidence limit.

Results

The parent compound was present in the blood plasma only in the samples collected 2 h after drug administration (mean 0.015 µg·ml⁻¹). Plasma concentrations of metabolites, expressed as the sum of FBZ-sulfoxide and FBZ-sulfone, reached their maximum in the samples collected 8 h after drug administration (mean 0.116 ± 0.029 µg·ml⁻¹) and declined to the levels below the detection limit by 60 h. The mean plasma concentration profile for the sum of the parent compound and metabolites is presented in Fig.1.

Peak plasma concentrations of metabolites (Cmax) was 0.133 µg·ml⁻¹, time to peak plasma concentrations (Tmax) was 17.0 h, and mean residence time (MRT) was 18.22 h. The total area under the plasma concentration versus time curve (AUC) was 3.5 µg·h/ml.

Pharmacokinetic parameters obtained from the sum of FBZ, FBZ-sulfoxide and FBZ-sulfone in pig plasma are shown in Table 1.

In the samples of tissues (kidneys, liver and muscles) no parent drug was found, whereas the minute amounts of metabolites (well below MRLs) were determined in pig’s liver on day 4 following a single oral administration of the drug.

Discussion

The results of this study showed that after oral administration of Fenbesan to pigs in the dose of 5 mg·kg⁻¹ b.w., fenbendazole was rapidly absorbed from the alimentary tract and metabolized to its sulfoxide and sulfone. The parent compound (FBZ) was detected only in the samples of plasma collected 2 h after drug administration. This corresponds to the results of the study described by Petersen and Friis (10) who detected $T_{\text{max}}$ for fenbendazole equal 3.75 h.
Fig. 1. Mean plasma concentration of fenbendazole and metabolites (sum of FBZ, FBZ-sulfoxide and FBZ-sulfone) after single oral administration of fenbendazole (5 mg kg$^{-1}$) to pigs (n=8).
Table 1
Pharmacokinetic variables (mean ± SD) for FBZ-sulfoxide

### Table 1

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>FBZ concentration (ln mg l⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>8</td>
<td>7</td>
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<tr>
<td>12</td>
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<td>48</td>
<td>3</td>
</tr>
<tr>
<td>60</td>
<td>2</td>
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**Fig. 2.** Biexponential decay curve of fenbendazole (sum of metabolites) with computed confidence belts. Assessment of withdrawal time by the intersection of MRL-level with upper confidence limit.
and FBZ-sulfone (sum of metabolites) in the pig plasma after single oral administration of fenbendazole (5 mg/kg b.w.)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pigs (n=8)</th>
</tr>
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<tbody>
<tr>
<td>Dose, mg/kg b.w.</td>
<td>5.0</td>
</tr>
<tr>
<td>$C_{\text{max}}$, µg·ml$^{-1}$</td>
<td>$0.133 \pm 0.032$</td>
</tr>
<tr>
<td>$T_{\text{max}}$, h</td>
<td>$17 \pm 7.6$</td>
</tr>
<tr>
<td>AUC, µg·h/ml</td>
<td>$3.5 \pm 0.60$</td>
</tr>
<tr>
<td>$\text{Cl}$, ml/h·kg</td>
<td>$1.40 \pm 0.47$</td>
</tr>
<tr>
<td>$V_d$, ml/kg</td>
<td>$8.29 \pm 5.39$</td>
</tr>
<tr>
<td>$T_{0.5\beta}$, h</td>
<td>$4.30 \pm 0.22$</td>
</tr>
</tbody>
</table>

Metabolites of fenbendazole FBZ-SO and FBZ-SO$_2$ in plasma after FBZ administration appeared rapidly and their concentrations always exceeded FBZ levels. Maximum concentration ($C_{\text{max}}$) and $T_{\text{max}}$, time to $C_{\text{max}}$ of metabolites (sum of FBZ-SO and FBZ-SO$_2$), were $0.133$ µg·ml$^{-1}$ and 17.6 h, respectively (Table 1). The concentrations of metabolites were below the limit of quantification in plasma samples obtained 60 h after administration of fenbendazole.

The total AUC (sum of the AUC for FBZ, FBZ-SO and FBZ-SO$_2$) was $3.5\pm0.6$ µg·h·ml$^{-1}$ and was lower than those found by the other authors (10). This may be explained by differences in the entity of fenbendazole formulations used in both experiments and/or by discrepancies in the technique of the drug administration in those two studies (with feed before morning feeding in present study and suspended in water given to the animals 1 h after feeding – reported by other authors (10).

In all the tissue samples collected (kidneys, liver and muscles) no fenbendazole was detected, whereas minute amounts of metabolites (well below the MRL) were found only in swine liver on day 4 following a single oral administration of the drug. These data suggest that FBZ is absorbed rapidly, reaches the tissues and is then eliminated rapidly without accumulation in tissues. In the studies carried out on pigs, reported by JECFA (6), the first sampling data for tissue residues were 5 d and metabolites of fenbendazole have been cleared from tissues before first sampling occasion.

All these results of residue studies on pigs indicate that withdrawal time for fenbendazole formulations may be fixed shorter than 4 d after administration. Results obtained in the present study on residue elimination do not allow to calculate precise withdrawal time (as there are no values for muscles). Approximate withdrawal time calculated from concentrations in plasma obtained in this study is 40 h (Fig. 2). However, to provide more security for the consumers, 3-d withdrawal time may be advised.

References