CONCENTRATION OF CEFACETRIL IN MILK AFTER ITS INTRAMAMMARY ADMINISTRATION TO COWS WITH HEALTHY AND INFLAMMED MAMMARY GLAND

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Abstract

The aim of the study was to develop a sensitive analytical method that enables to determine cefacetril concentration in milk after its application to the udders, as well as to establish and compare cefacetril’s basic pharmacokinetic indicators after its intramammary application to cows with inflammed and healthy udders. The content of cefacetril was determined with the use of high-performance liquid chromatography with UV detection. Usefulness of the developed analytical method in the studies of pharmacokinetics of cefacetril has been demonstrated. Results obtained during the statistical analysis and bacteriological examinations allowed to regard the antibiotic as an accurately selected medical product used in the treatment of mastitis in cows.

Key words: cows, milk, mastitis, cefacetril, pharmacokinetics.

β-lactam antibiotics, including cefacetril (Fig. 1), have characteristic kinetics of antibacterial activity, dependent on time. It means that to obtain some antibacterial activity, the concentration of antibiotic should not decrease below an effective value for a possibly long period. It happens, because these antibiotics show only small post-antibiotic effect. If the concentration of drugs goes down below their effective values then bacteria begin to multiply very quickly. Comparatively small concentration of antibiotics causes optimum antibacterial activity and further elevation of the concentration does not lead to a significant increase in their activity power. From practical point of view it means that β-lactam antibiotics, including cephalosporin should be dosed so, that their levels do not decrease below effective values. It demands possibly frequent medicating or use of prolonged release medicines. The requirement of clinical efficiency of β-lactam is to hold effective antibacterial concentration for at least 40% of period between foregoing doses (2-5).

Metabolism of cephalosporins takes place in the liver, where these antibiotics undergo deacetylation to less active metabolites as a result of splintering of acetoxy methylc groups at C3. Pharmacokinetics after parenteral use of cefacetril in most animals (rats, rabbits, cattle, sheep, goats) and man (1) is characterised by low distribution (Vd from 0.2 to 0.5 /kg) and by short period of biological half-life – less than one hour.

Antibiotics from cephalosporin group are usually excreted in large concentrations with urine, both by glomerular filtration and active tubular secretion (1-4).

Some cephalosporins are excreted along with bile e.g.: cepazolin, cephamandol, cephameron, or cephamikson. Cefacetril is excreted in 80% with urine within 6 h from the moment of administration. Antibiotic being found in urine is deacetylated in 25%. After use of 1 g i.v., renal clearance is equal to 313 mL/min; excretion of cefacetril is suppressed by probenecid (dipropyloamid acid p-carboxy-benzensulphonic), which stops backward absorption of urinary acid what indicates participation of tubular secretion in elimination of this antibiotic. In the kidneys, incapacity excretion is free. This medicine is concentrated in bile in a small amount; after application of 1 g of cefacetril, its concentration in bile is equal to about 1.9 mg/L in 4 h (5, 6, 8, 10, 14-17).

Fig. 1. Structural formula of cefacetril.

Nowadays existing researches concerning pharmacokinetics of cephalosporin admitted to use in dairy cattle have been carried out to a large extent by means of biological methods. These tests based on the standard – pattern tribes are characterised by too little
sensitivity to detect the remains of the antibiotics in milk. Moreover, other substances in milk can stop the growth of used test microorganisms. High performance liquid chromatography (HPLC) is the method recommended by CVMP and Experts Committee WHO/FAO for the determination of cephalosporin in cow milk as the method showing a suitable sensitivity (9, 11-14, 18, 19).

The aim of the study was to determine the concentration of cefacetril in milk after its intramammary application and to compare its pharmacokinetics in healthy and mastitic cows.

Material and Methods

Animals and medicine. Research has been conducted on 18 dairy cows, Polish Black and White breed, 4-12 years of age. The animals were treated with the intramammary preparation Masticif (Biowet, Poland) containing 250 mg of cefacetril in 5 ml (8 g). The cows were allotted to two groups: group A comprised eight healthy cows being in a final phase of lactation and group B containing ten cows with inflammation of lactating mammary gland.

Animal treatment. Before application of the antibiotic and after careful washing and disinfection of the mammary gland, the samples of milk had been taken from all cows (test 0). Then cefacetril in the form of intramammary reconstitution had been applied by a turbo syringe in the same manner in both groups.

Milk sampling. After the application of cefacetril, the udder was carefully massaged in order to distribute better and faster the medicine and to allow the mammary gland to absorb it. Milk samples from the antibiotic-treated udders were taken 2 h, 3 h, 6 h, 8 h, 10 h, 24 h, 36 h, and 48 h after medicine application. In case of cows with inflammable mammary gland, the milk samples were taken before and after the end of the therapy. The programme of the study was accepted by the Local Ethical Committee for Researches on Animals.

Chromatography. Chromatography method according to Daeseleire et al. (7), in own modification (addition of 1 ml 70% of trichloroacetic acid on order to precipitate protein), became initially checked in range of linearity, accuracy, detection limits (LOD=0.71 µg/mL), markings (LOQ=2.17 µg/mL), and specificity and precision. The conducted validation confirmed usefulness of applied procedures to determine the level of cefacetril in milk. The process was conducted on high-performance fluid chromatograph (Gilson, USA) with the use of column LiChroCART 125x3 mm, Purospher RP - 18e (5 mm), movable phase - 0.1 M KH₂PO₄, pH 3.0, acetonitril (90:10, v/v), as well as UV detection at about 254 nm of length wave.

Cefacetril extraction from milk samples. One milliliter of 70% trichloroacetic acid was added to 3 ml of milk samples. Then the samples were mixed for 2 min on Vortex. Next, the samples were centrifuged for 10 min at 6,000 x g. After protein precipitation and centrifuging, the samples were filtered through a 0.2 µm teflon syringe filter to remove possible remaining contamination. From such prepared samples, 20 µl was taken for HPLC analysis and dosed onto the chromatography column.

Statistical analysis. To conduct the statistical analysis of the results, the computer programme Statistica 6.0 was used. The average values (x) and standard deviations (±SD) of cefacetril concentration in milk of cows from groups A and B were presented in Table 1. Relevance of differences among averages in following hours after application of the medicine in group of healthy cows and in cows with mammary gland inflammation were verified by means of t-Student test (α=0.05). Similar procedure was applied in case of pharmacokinetic coefficients.

Results

Average concentrations of cefacetril in milk from healthy (group A) and inflammable (group B) lactating mammary glands, after a single intramammary application of Masticif (250 mg of cefacetril) are presented in Table 1. The highest concentrations of cefacetril in both groups of cows were detected 2 h after its application and amounted to 115.30 µg/mL in group A and 237.31 µg/mL in group B. A significant decrease in the concentration of cefacetril in both groups of animals was observed 6 h after its application. The concentration in cows from group A was 25.35 µg/mL and in cows from group B – 28.45 µg/mL. In the following hours, the concentration of cefacetril was still decreasing and after 24 h, it was 1.57 µg/mL in group A and 2.15 µg/mL in group B.

On the basis of the obtained concentrations of the tested antibiotic, in the next step of the analysis, basic pharmacokinetic parameters connected with speed of cefacetril elimination from milk were defined using computer programme PK Solution 2.0. The parameters showed that cefacetril applied in cows from group A got lower maximum concentrations in milk (Cmax=140.50 µg/mL) than in animals from group B (Cmax =174.29 µg/mL).

Field values of surface under curve (AUCµg/h/mL) were also lower - set for healthy animals (group A) – 619.94, in comparison to animals from group B – 1,022.96. Inversely, MRT value for cefacetril in cows with mastitis seemed to be higher – 119.60 h, in comparison to healthy cows – 22.20 h. The volume of distribution of the medicine (Vd) in cows from group A was 1.19 mL/kg, while in cows from group B it was comparatively higher – 2.78 mL/kg. Values of pharmacokinetics coefficients were shown in the Table 2.

Relevance estimation of differences between average values of compared pharmacokinetics coefficients in cows from groups A and B showed essential differences between pairs of the following pharmacokinetics coefficients: Cmax, AUC, MRT, and Vd. Lack of essential differences was found only in case of - Tmax (Table 2).
Table 1
Average concentration of cefacetril (µg/mL) in milk after its intramammary application (250 mg) to dairy healthy cows and cows with inflammation of the mammary gland at individual temporary points after the antibiotic application

<table>
<thead>
<tr>
<th>Animals</th>
<th>2 h</th>
<th>3 h</th>
<th>6 h</th>
<th>8 h</th>
<th>10 h</th>
<th>24 h</th>
<th>36 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy cows (n=10)</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>115.3* ± 3.06</td>
<td>71.5 ±47.8</td>
<td>25.3 ±19.9</td>
<td>12.5 ±14.5</td>
<td>7.9 ±7.5</td>
<td>1.5 ±0.5</td>
<td>1.3 ±0.3</td>
<td>1.1 ±0.2</td>
</tr>
<tr>
<td>Mastitic cows (n=8)</td>
<td>237.3* ±175.5</td>
<td>85.4 ±87.0</td>
<td>28.9 ±28.4</td>
<td>16.3 ±18.3</td>
<td>11.1 ±16.9</td>
<td>2.3 ±2.1</td>
<td>0.8 ±0.4</td>
<td>0.8 ±0.4</td>
</tr>
</tbody>
</table>

* P≤0.05 ; ± - SD.

Table 2
Pharmacokinetic coefficients of milk according to health condition

<table>
<thead>
<tr>
<th>Animals</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC (µg/h/mL)</th>
<th>MRT (h)</th>
<th>V&lt;sub&gt;d&lt;/sub&gt; (mL/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy cows (n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x ±SD</td>
<td>140.5 ±27.49</td>
<td>2.0 ±0.0</td>
<td>619.94 ±141.46</td>
<td>22.2 ±12.46</td>
<td>1.19 ±0.37</td>
</tr>
<tr>
<td>RSD (%)</td>
<td></td>
<td>0.0</td>
<td>22.81</td>
<td>56.12</td>
<td>31.84</td>
</tr>
<tr>
<td>Mastitic cows (n=10)</td>
<td></td>
<td>174.29 ±38.53</td>
<td>2.0 ±0.0</td>
<td>1,022.96 ±465.39</td>
<td>119.60 ±79.53</td>
</tr>
<tr>
<td>x ±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD (%)</td>
<td></td>
<td>22.1</td>
<td>45.49</td>
<td>66.49</td>
<td>46.94</td>
</tr>
</tbody>
</table>

* P≤0.05.

Discussion

The obtained concentrations of cefacetril in milk within the first hours after application of the antibiotic testify its good pharmacokinetic characteristic, which suggests its usefulness in the treatment of inflammation of the mammary gland. Attained concentrations of antibiotic are many times higher than MIC values for pathogenic microbes causing mastitis and allow an efficient elimination of pathogenic factors.

In spite of the fact that cefacetril was not determined in blood (lack of agreement of owners to conduct repeated tests), conducted study pictured very distinctly the concentrations of this antibiotic in the udder after its intramammary application. Detectable levels of cefacetril in milk from cows of both groups were observed 48 h after its application. It allows assuming that independently from gland condition (health and mastitic), the main distribution space after the intramammary application of cefacetril is the mammary gland because of a strong blood-udder-barrier in both experimental groups. This was confirmed by test results with labelled cefacetril (¹⁴C), which showed that 54.6% of this antibiotic is recovered from milk and only 21% with urine (1). In the same report, it was stated that after two applications of cefacetril (250 mg/) into the mammary gland, the antibiotic was detected 5 d after the second application only in tissue of lactating gland. The pharmacokinetics analysis of cefacetril confirmed that the main distribution space for this drug is the mammary gland, and inflammation state seems to limit this process only to udder, even to the infected quarter.

References