TETRACYCLINE CONCENTRATIONS IN BLOOD AND MILK OF COWS FOLLOWING INTRAUTERINE TREATMENT OF ACUTE OR SUBACUTE/CHRONIC ENDOMETRITIS

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The tetracycline hydrochloride (TTC) kinetics in serum and elimination from milk were evaluated in cows treated with this antibiotic in the form of intrauterine pessaries. The antibiotic was administered to 3 cows with acute endometritis (AEM) at a dosage of 3 g per animal and to 3 cows with subacute/chronic endometritis (SCHEM) at a dosage of 2 g per animal. Blood and milk samples were collected before treatment and at 12 h intervals for 6 consecutive days. The samples were analysed by HPLC method with solid phase extraction. After 12 h, the mean concentrations of the drug in serum were 206.6 ± 29.0 µg.l⁻¹ for the AEM group and 210.3 ± 112.4 µg.l⁻¹ for the SCHEM group. Concentrations of the antibiotic in milk at the same time were 227.7 ± 128.1 µg.l⁻¹ and 227.8 ± 35.8 µg.l⁻¹ for the AEM and SCHEM groups, respectively. The residual levels of the drug in milk: < 100, < 30, and 0 µg.l⁻¹ were obtained in 64, 116, and 144 h for the AEM group, and in 52, 86, and 120 h for the SCHEM group. The results demonstrate that TTC administered as a solid form is faster absorbed from the uterus in moderate and mild forms of endometritis than in severe ones.

Key words: cows, endometritis, tetracycline, residues, milk.

Puerperal endometritis/metritis, whether primary or following a retained placenta is considered to be a common disease, which has an adverse influence on the reproduction performance of dairy cows. Based on the clinical symptoms and the time after parturition in cows, we can distinguish acute putrid (endo) metritis, which occurs within 14 d after parturition and later subacute/chronic endometritis (7, 21). These clinical forms are more frequent in dairy farms with subsequent intrauterine antibiotic therapy. Various treatment have been used in an attempt to reduce the sequel of
retained placenta and inflammation of the uterus. Intrauterine administration of tetracycline has been recommended because of its broad spectrum of antibacterial activity in the presence of organic material (1, 3, 12).

Residues of antibiotics were studied after parenteral administration (2, 5, 6, 13, 14, 16, 17, 19, 20). Concentrations of oxytetracycline in the plasma of healthy cows are considerably higher than those found in animal affected by metritis. In this case, the absorption of oxytetracycline from the uterus seems to be influenced by the degree of uterine inflammation.

The aim of the study was the determination of the resorption of tetracycline and its elimination from serum and milk after a single intrauterine administration of a therapeutic dose in dairy cows suffering from acute and subacute/chronic inflammation of the uterus.

**Material and Methods**

The trial was conducted on six dairy cows (Lowland black-and-white and Slovak white-and-brown). The cows were housed in the stalls under common practical conditions and were fed on maize silage and cereal-base concentrate. The cows were milked in their stalls twice a day into a calibrated glass container.

According to the vaginal examination and character of vaginal discharge the animals were divided into two groups. In the first group (n=3) there were cows suffering from acute puerperal endometritis (AEM). Endometritis was diagnosed on the basis of cervical discharge of pathologically changed lochia. Pathological lochia was thin to watery with a dirty-redish colour and a strong odour. The animals were treated once with 3 g of tetracycline hydrochloride pessaries (Lečiva Praha, Czech Republic). The second group (n=3) consisted of cows with subacute/chronic endometritis. The animals were more than 14 d after parturition and had purulent or mucopurulent (approximately 50% pus) mucus with flecks of pus (<50% pus) cervical discharge. The cows in this group were treated once with 2 g of tetracycline pessaries.

The blood samples were taken from the jugular vein of each cow before the treatment (0 h) and at 12 h intervals up to 144 h after the treatment. The tubes were left to stand for approximately 0.5 h for clotting before being centrifuged at room temperature for 15 min. The blood serum was recovered and frozen at -20°C for future analysis.

The milk samples were collected from the bulk milk of each cow at the same intervals as the blood. The samples were immediately frozen and stored until the analysis.

Tetracycline levels in serum and milk were determined by the HPLC method with solid-phase extraction with LOD 10 ng/g and recovery about 85% (14, 18).

All the data were statistically analysed using computer software GPPP, Version 2.01, 1996. The differences were evaluated by the Student t-test.

**Results**

Absorption and depletion of TTC in blood of cows suffering from AEM and SCHEM are presented in Figs 1 and 2, respectively. Absorption from the uterus to the peripheral blood shows maximum concentrations in all cows after 12 or 24 h. Data of
absorption of TTC from the uterus into peripheral blood and elimination from milk are reported in Figs 3 and 4, respectively. Antibiotic elimination from serum and milk of cows with SCHEM were shorter than that in the AEM group. The antibiotic concentration below LOD in the SCHEM group occurred at 48 h after treatment, but in AEM cows 60 h later. The maximum individual concentrations of TTC in milk ranged between 164.9 and 458.6 µg.l⁻¹ for AEM cows (Table 1). Mean maximum concentration of the drug in milk of the AEM group was 276.4 ± 138.2 µg.l⁻¹ (Table 1); the maximum was reached at the 24th h. Individual maximum concentrations in milk in the SCHEM group ranged between 159.3 and 719.7 µg.l⁻¹ (Fig. 6), the maximum was reached at the same time as in the AEM cows (Fig. 5). Mean maximum concentration of antibiotic in the SCHEM group was slightly higher than in the AEM group: 369.6 ± 314.8 µg.l⁻¹ (Table 1), but this difference was not significant.

The tetracycline residues in milk according to different “safe levels” of the drug are reported in Table 2. After a single intrauterine treatment with TTC pessaries, the MRL level (< 100 µg.l⁻¹) the cows in the AEM group occurred 12 h later than in the SCHEM group. The “harder criterion” level (< 30 µg.l⁻¹) was also achieved by the AEM later than in the SCHEM group (difference 28 h).

**Table 1**

Maximum tetracycline concentrations (µg.l⁻¹) after intrauterine treatment of cows with the inflammation of the uterus

<table>
<thead>
<tr>
<th>Concentration Variables</th>
<th>Serum</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AEM*</td>
<td>SCHEM*</td>
</tr>
<tr>
<td>Min</td>
<td>167.8</td>
<td>149.7</td>
</tr>
<tr>
<td>Max</td>
<td>384.2</td>
<td>245.0</td>
</tr>
<tr>
<td>Mean</td>
<td>210.4</td>
<td>206.6</td>
</tr>
<tr>
<td>SEM</td>
<td>194.7</td>
<td>50.3</td>
</tr>
</tbody>
</table>

* - AEM – acute endometritis; SCHEM – subacute/chronic endometritis

Differences are not significant
Table 2
Duration (h) of tetracycline residues in milk after intrauterine treatment of cows with inflammation of the uterus

<table>
<thead>
<tr>
<th>Concentration Variables</th>
<th>Acute endometritis</th>
<th>Subacute/chronic endometritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100 µg.l⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>64.0</td>
<td>52.0</td>
</tr>
<tr>
<td>SEM</td>
<td>13.9</td>
<td>18.3</td>
</tr>
<tr>
<td>Max</td>
<td>72.0</td>
<td>72.0</td>
</tr>
<tr>
<td>&lt;30µg.l⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>116.0</td>
<td>88.0</td>
</tr>
<tr>
<td>SEM</td>
<td>25.0</td>
<td>30.2</td>
</tr>
<tr>
<td>Max</td>
<td>144.0</td>
<td>120.0</td>
</tr>
</tbody>
</table>
Fig. 1. Individual concentrations of tetracycline (µg.l\(^{-1}\)) in blood serum of cows with acute endometritis.
**Fig. 2.** Individual concentrations of tetracycline (µg.L⁻¹) in blood serum of cows with subacute/chronic endometritis
Fig.3. Individual concentrations of tetracycline (µg.l⁻¹) in milk of cows with acute endometritis.
Fig. 4. Individual concentrations of tetracycline (µg.l$^{-1}$) in milk of cows with subacute/chronic endometritis
**Fig. 5.** The mean concentrations of tetracycline (µg/l) in serum and milk from cows with acute endometritis after intrauterine treatment.
Fig. 6. The mean concentrations of tetracycline (µg/l) in serum and milk of cows with subacute/chronic endometritis after intruterine treatment.
Discussion

Generally, in cows with an inflammation of the uterus, the intrauterine treatment with 2 to 3 g of tetracycline is recommended (1). The higher dose is recommended in severe forms of endometritis. The absorption of TTC from the uterus into the peripheral circulation occurs within 12 h. The rate of the absorption depends on the application forms of antibiotic (spray, solution or solid forms – pessaries) (2, 9, 15). The rate of penetration of TTC from the uterus into peripheral blood after treatment with solid forms (pessaries) indicated, that the maximum levels of antibiotic were determined in the first samples (after 12 h). The use of different doses of antibiotic did not cause significantly higher concentrations of TTC in serum of the first samples (12 h, Figs 5 and 2).

The rate of penetration of TTC from the uterus into peripheral blood after treatment with solid forms (pessaries) indicated, that the maximum levels of antibiotic were determined in the first samples (after 12 h). The use of different doses of antibiotic did not cause significantly higher concentrations of TTC in serum of the first samples (12 h, Figs 5 and 2).

The content of TTC in the serum of AEM cows lasted on average 68.0 ± 27.7 h, but in the SCHEM group only 28.0 ± 6.9 h with detectable levels within 36 h and 84 h for AEM and SCHEM group, respectively. Roncada et al. (16) in the study on comparable doses and applications forms in healthy cows found residual content of antibiotics up to days 2 and 3, but at 24 h collection intervals. Other authors (2) found greater absorption of the drug from healthy postpartum uterus.

Our differences in absorption and residual content of TTC in blood of cows with AEM and SCHEM support the results of Righter et al. (15) who concluded that TTC level in serum is clearly lower in the subject treated on the 2nd d after parturition than in animals treated later (12-32 d post partum).

Absorption of antibiotic from the uterus to peripheral blood was reflected on its level in milk. Using less sensitive methods (Bacillus stearothermophilus Disc Assay), Haaland et al. (11), with the same doses of antibiotic, but in infusion forms, found that most samples were free of residues by 84 h. Higher doses (5 g) of antibiotic in infusion form in cows with retained placenta (6) had residual effects on average 52.3 h (max. 144 h). The residual content of TTC in milk of healthy cows treated with the same doses as in our experiment (3 g of intrauterine pessaries) was not determined 72 h (3/5 animals) and 84 h (5/5 animals) (16). The duration of residues in milk may be dependent on such factors as the relative blood flow to the myometrium, the amount of antibiotics expelled via vagina through open cervix, contractions of the uterus, amounts of fluid in the uterus or milk yield.

The residual limit of TTC in cows’ milk < 100 µg.l⁻¹ in our experiment was obtained on average after 64 h in cows with AEM and after 52 h in cows with SCHEM. Maximum residual time at this level in some cows was 72 h. After this period, TTC concentrations were below the maximum residual limit. The withdrawal time for the milk laid down by the drug producer is 96 h. Current maximum residual limit of TTC in cows’ milk in EC legislation (4) and also in Slovak Codex Alimentarius (8) is 100 µg.l⁻¹, but recently published “safe limit” which was announced by FDA is 30 µg.kg⁻¹ (10).

The results of this study indicate, that absorption and elimination from milk of intrauterine tetracycline pessaries administered to two group of cows with AEM and SCHEM had individual characters. The maximum time to reach MRL limits is obtained after 144 and 120 h in cows suffering from AEM and SCHEM, respectively. Current officially established MRL (100 µg.l⁻¹) is reached in some cows after 48 h, but mostly after 72 h.
References
