Pharmacokinetics and residual behaviour in milk of oxytetracycline in cows following administration of uterine pessaries

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The plasma kinetics and residual depletion in milk of cows treated by the intrauterine route with pessaries containing oxytetracycline (OTC) were evaluated. The antibiotic was administered to five healthy Friesian cows at a dosage of 3g/head in the early *post partum* phase. Blood samples were collected before and at different time intervals (3, 6, 12, 24, 48, 72, 84, and 96 h) after treatment. Milk was drawn before treatment and at 12-h intervals for 4 consecutive days.

Samples were analysed by a high-performance liquid chromatography method and the pharmacokinetic parameters were processed using the minimum Akaike information criterion estimation (MAICE) test. The mean values obtained indicated a relatively low area under the concentration time curve (25.19 ± 12.61 μg/mg per h) and maximum plasma concentration (C_{max}) (0.549 ± 0.278 μg/mL) with delayed time to C_{max} (11.71 ± 4.15 h) and elimination half-life (21.96 ± 4.42 h).

A similar pattern could be shown for milk, in which measurable residual levels are found in two out of five animals until the 72nd hour after treatment. Data obtained demonstrate that OTC administered as a solid form is poorly and slowly absorbed from the uterus of cows.

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INTRODUCTION

Oxytetracycline (OTC) is a broad spectrum antibiotic and is indicated for the treatment and control of infections caused by, or associated with OTC-sensitive, rapidly growing microorganisms (Huber, 1988). Its antibacterial efficacy against many infections caused by Gram-positive and Gram-negative bacteria is well documented. The antibiotic may also be used by the intrauterine route (Seguin *et al.*, 1974; Oxender & Seguin, 1976; Miller & Bergt, 1976). However, although this drug is not approved by the FDA for use in lactating dairy cattle, other than for low-dosage oral administration (Norell & Packham, 1992; Anderson *et al.*, 1995), it could be shown that intrauterine administration represents a useful therapy, especially in the treatment and prophylaxis of *post partum* endometritis in the cow. Endometritis implies inflammation of the endometrium, and is clinically characterized by the presence of a mucopurulent vulval discharge 21 days or more after calving (Sheldon & Noakes, 1998). The direct intrauterine administration of OTC produces immediate therapeutic concentrations in the caruncles and endometrium of both healthy and affected animals, and because of its relatively low absorption into the bloodstream, the therapeutic action is largely confined to the uterine lumen and endometrium (Bretzlaff *et al.*, 1983). Tetracyclines are known to be active under anaerobic conditions and are only partly inactivated by the purulent material, cell debris and pH levels found in affected uteri (Cairoli *et al.*, 1993). On the contrary, the passage of OTC from the uterine lumen into the bloodstream seems to be influenced by the physiological or pathological condition of the treated animals. In fact, OTC concentrations found in plasma of healthy cows are considerably higher than those found in the animals affected by metritis

(Bretzlaff *et al.*, 1983). In this case, the absorption of OTC from the uterus seems also to be influenced by the time interval between parturition and the administration of the antibiotic: this is indirectly underlined by the fact that in cows affected by metritis, the drug can be detected in milk for a longer period when administered in the early *post partum* phase than in the later periods (Kaneene *et al.*, 1986; Girardi, 1991).

Moreover, Girardi *et al.* (1990b) and Girardi (1991) observed a remarkable variability of the absorption of the antibiotic, after intrauterine administration, in relation to the vehicle used. These studies emphasized that the OTC passage through the uterine mucosa is strongly influenced by the carrier.

In the present study, the antibiotic levels in serum and milk of healthy dairy cows, treated in the *post partum* phase by the intrauterine route with a solid formulation (intrauterine pessaries) have been investigated to evaluate OTC pharmacokinetics and its residual behaviour in milk.

MATERIALS AND METHODS

Animals and treatment

The trial was conducted on five Friesian dairy cows, with a body weight of 500–650 kg. The cows were kept indoors in an experimental animal shed reproducing the standard operating husbandry. They were fed with concentrated feed and hay, and received water *ad libitum*. All animals had a normal calving delivery, were clinically healthy and had not been treated with antibiotics in the 30 days before the trial.

For the study, pessaries containing 1.08 g of OTC hydrochloride containing 1 g of OTC base, (Gelbiox®, FARMACEUTICI GELLINI S.p.A., Aprilia (LT), Italy) were used.

Each cow was treated once with three pessaries (3 g OTC/animal) by the intrauterine route 24 h after parturition.

Sampling

Immediately before treatment (0 time), and then at different intervals, blood samples were collected from the jugular vein (3, 6, 12, 24, 48, 72, 84 and 96 h after treatment) from each animal. Then blood was centrifuged immediately to obtain serum.

Milk samples were collected from bulk milk of each animal before drug administration and at 12, 24, 36, 48, 60, 72, 84 and 96 h after treatment. Serum and milk samples were frozen promptly and stored at -20 °C until analysis.

Analytical procedures

Extraction and cleaning up. Two mL of serum were buffered to pH 6 with 2.5 mL of 0.2 M phosphate buffer (pH 5) and then shaken by vortex. Samples were purified on 500 mg SPE-C18 columns (J.T. Baker Inc., Phillipsburg, NJ, USA), previously activated with 2 mL methanol and 2 mL water:methanol 99:1 v:v. After being washed with the same mixture (1 mL) and under addition of 400 μL methanol:0.1 M hydrochloric acid (2:1 v:v), the antibiotic was eluted from the columns using this latter solution (1 mL).

The extraction of OTC from milk was carried out by using the same type of column, which was activated according to the procedure previously described. In this case, after the addition of the sample (2 mL), the column was washed twice, first with water (2 mL) and then with a mixture containing water:methanol 70:30 v:v (2 mL).

Finally, OTC was eluted with 2 mL of methanol:0.1 M hydrochloric acid (2:1 v:v).

High-performance liquid chromatography. Aliquots (50 μL) of the eluates were analysed by using a partially modified high-performance liquid chromatography (HPLC) method described by Oka *et al.* (1987).

Briefly, a Beckman (San Ramon, CA, USA) 116 pump, a Beckman 507 automatic sampler and a UV-Diode Array Beckman 168 detector, all connected to an IBM PS2/50, equipped with a software GOLD release 4.0 and an EPSON FX850 printer were used. The analysis was carried out under the following operational conditions:

- Column: Ultracarb 5 ODS 150 × 4.6 mm plus a 5 ODS pre-column 30 × 4.6 mm (Phenomex; Chemtek Analytica, Anzola Emila, BO, Italy)
- Eluting mixture: CH_3CN:H_3PO_4 (0.01 M) 15.5:84.5 (v:v)
- Flux: 1.2 mL/min.
- Wavelength: 350 nm

Under the above-mentioned conditions, OTC is characterized by a retention time of about 2.9 min.

On the basis of suitable calibration curves, previously set up with solutions at decreasing concentrations of OTC hydrochloride standard (range: 0.05–10 μg OTC/mL), recoveries were $83 \pm 0.5\%$ for serum and $81 \pm 0.6\%$ for milk. The precision and accuracy of the analytical method were evaluated by repeating the analysis on serum and milk samples on different days. This allowed determination of mean coefficients of variation equivalent to 3.09% (serum) and 3.44% (milk). The detection limit for both serum and milk was 0.05 μg/mL.

Evaluation of the data

The pharmacokinetic analysis of the data obtained was conducted by the minimum Akaike information criterion estimate (MAICE) test (Yamaoka *et al.*, 1978), using the following equation: $C = Y_2 \exp(-\lambda_2 t) - Y_1 \exp(-\lambda_1 t)$, which better describes the pharmacokinetic pattern of serum concentrations (C) over time (t) of OTC administered by the intrauterine route. The initial estimates of the Y_1 and Y_2 coefficients were obtained by means of the method of residuals, which is a commonly used technique for resolving a curve into its various exponential components, while λ_1 and λ_2 were calculated on the basis of the sampling points (λ = slope of the respective phase) (Gibaldi & Perrier, 1982).

By using a program based on the Marquandt algorithm, it was possible to adapt the equation to the data obtained from each animal.

RESULTS

OTC serum concentrations and the pharmacokinetic parameters of cows treated with intrauterine pessaries (3 g OTC/animal) are reported in Tables 1 and 2, respectively.

Data obtained from the present study show that there was passage of the antibiotic from the administration site (uterus) to the bloodstream in all animals. The serum concentration pattern revealed an increase in the antibiotic levels until the 12th hour (0.547 ± 0.264 μg/mL) after treatment, followed by a progressive decrease characterized by a constant detection of amounts of antibiotic up to the 48th hour after treatment (0.255 ± 0.150 μg/mL). Detectable levels of OTC were still found up to the 84th hour (0.065 ± 0.014 μg/mL) in two out of five subjects (Table 1).

The mean values of the calculated pharmacokinetic parameters show that the maximum concentration of OTC ($C_{max} = 0.549 \pm 0.278$ μg/mL) was reached within 11.71 ± 4.15 h (t_{max}), the depletion phase was characterized by an elimination half-life ($t_{1/2}\beta$) of 21.96 ± 4.42 h and that the area under the curve (*AUC*) was 25.19 ± 12.61 μg/mL per h (Table 2).

Data of residual depletion of OTC in milk are reported in Tables 3 and 4.

An increase in the drug concentrations up to the 2nd milking (24 h after treatment) could be shown for all subjects, with values ranging from 0.090 to 0.385 μg/mL. Subsequently, a gradual decrease led to values constantly below the detection limit of the method, starting from the 84th hour after treatment (Table 3).

From a pharmacokinetic point of view, the maximum antibiotic concentration in milk was 0.209 ± 0.103 μg/mL and was reached within 18.36 ± 1.11 h. The $t_{1/2}\beta$ was 16.33 ± 2.06 h and the *AUC* presented a mean value of 10.6 ± 4.88 μg/mL per h (Table 4).

DISCUSSION

On the basis of the results obtained during the present trial, we can affirm that OTC is poorly absorbed from the uteri of cows treated with a dosage of 3 g OTC/cow, administered as pessaries 24 h after parturition. This statement is supported by the maximum blood concentrations (C_{max}) (0.549 ± 0.278 μg/mL) and by the *AUC* value (25.19 ± 12.61 μg/mL) reached by the antibiotic.

Although it is not possible to compare directly the data reported by Righter *et al.* (1975), following intrauterine infusion of OTC at the dose of 500 mg/cow, the results obtained by the authors highlight that the antibiotic absorption rate increases according to the time elapsed from parturition. In fact, OTC concentrations in serum of *post partum* cows are clearly lower in subjects treated on the 2nd day after parturition than in the animals treated in the subsequent phases (12, 22, 32 days *post partum*).

Table 1. Oxytetracycline (OTC) concentrations (μg/mL) detected in cows' serum after administration of intrauterine pessaries at a dosage of 3 g/head

Cow	Time after treatment (h)								
	0	3	6	12	24	48	72	84	96
1	<0.05	0.585	0.811	0.863	0.740	0.361	0.187	0.075	<0.05
2	<0.05	0.263	0.371	0.779	0.682	0.457	0.157	0.055	<0.05
3	<0.05	0.150	0.206	0.236	0.132	0.095	<0.05	<0.05	<0.05
4	<0.05	0.282	0.368	0.456	0.342	0.208	0.115	<0.05	<0.05
5	<0.05	0.318	0.387	0.401	0.289	0.155	0.061	<0.05	<0.05
Mean ± SD	<0.05	0.320 ±0.161	0.429 ±0.226	0.547 ±0.264	0.437 ±0.262	0.255 ±0.150	0.130* ±0.055	0.065** ±0.014	<0.05

*Mean calculated on four positive values. **Mean calculated on two positive values.

Table 2. Pharmacokinetic parameters for OTC in cows' serum after administration of intrauterine pessaries at a dosage of 3 g/head

Parameters	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Mean ± SD
C_{max} (μg/mL)	0.913	0.750	0.219	0.444	0.417	0.549 ± 0.278
t_{max} (h)	10.894	18.810	9.07	11.35	8.42	11.71 ± 4.15
$K\alpha$ (h^{-1})	0.2014	0.06099	0.2905	0.202	0.2993	0.211 ± 0.096
$K\beta$ (h^{-1})	0.0313	0.04729	0.02667	0.02786	0.0312	0.0329 ± 0.0083
$t_{1/2}\beta$ (h)	22.071	14.6547	25.981	24.8762	22.2143	21.959 ± 4.42
AUC (μg/mL per h)	39.535	37.014	10.457	21.561	17.404	25.194 ± 12.61

C_{max}, highest plasma concentration; t_{max}, time at which C_{max} occurs (h); $K\alpha$, first-order absorption rate constant; $K\beta$, first-order elimination rate constant; $t_{1/2}\beta$, half-time of the first-order elimination rate constant; *AUC*, total area under the plasma drug concentration versus time curve.

Table 3. OTC concentrations (μg/mL) detected in cows' milk after administration of intrauterine pessaries at a dosage of 3 g/head

Cow	Time after treatment (h)								
	0	12	24	36	48	60	72	84	96
1	<0.05	0.328	0.385	0.310	0.160	0.091	0.075	<0.05	<0.05
2	<0.05	0.145	0.155	0.139	0.109	0.062	0.058	<0.05	<0.05
3	<0.05	0.082	0.090	0.078	0.053	<0.05	<0.05	<0.05	<0.05
4	<0.05	0.193	0.219	0.185	0.124	0.065	<0.05	<0.05	<0.05
5	<0.05	0.176	0.185	0.152	0.111	0.054	<0.05	<0.05	<0.05
Mean ± SD	<0.05	0.185 ± 0.091	0.207 ± 0.110	0.173 ± 0.086	0.111 ± 0.0385	0.068* ± 0.016	0.066** ± 0.012	<0.05	<0.05

*Mean calculated on four positive values. **Mean calculated on two positive values.

Table 4. Pharmacokinetic behaviour of OTC in cows' milk after administration of intrauterine pessaries at a dosage of 3 g/head

Parameters	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Mean ± SD
C_{max} (μg/mL)	0.373	0.162	0.094	0.225	0.191	0.209 ± 0.103
t_{max} (h)	17.69	20.29	17.80	17.71	18.32	18.36 ± 1.11
$t_{1/2}\beta$ (h)	14.82	19.91	15.33	15.44	16.13	16.33 ± 2.06
AUC (μg/mL per h)	18.17	9.43	4.63	11.03	9.76	10.60 ± 4.88

C_{max}, highest milk concentration; t_{max}, time at which C_{max} occurs (h); $t_{1/2}\beta$, half-time of the first-order elimination rate constant; *AUC*, total area under the milk drug concentration versus time curve.

Girardi *et al.* (1990a) observed that OTC is not well absorbed in cows affected by a mild metritis and treated by the intrauterine route with doses of 6–6.6 mg/kg in a spray formulation, as emphasized by the *AUC* value (12.94 ± 4.98 μg/mL per h). This is also supported by Bretzlaff *et al.* (1983), who observed that, following intrauterine infusion of 5.5 mg/kg b.w. OTC to healthy cows, the drug absorption rate ($F = 0.5 \pm 0.23$) was higher than in cows affected by metritis ($F = 0.23 \pm 0.03$) and treated with the same dosage.

In the present study, the time needed for OTC to reach peak blood concentrations (t_{max} 11.71 ± 4.15 h) was longer than that reported by other authors; in particular: 2–4 h (Bretzlaff *et al.*, 1983), 6–8 h (Righter *et al.*, 1975), and 8 h (Girardi *et al.*, 1990a). This observation may be partially explained by the time the formulation takes to dissolve. In fact, unlike the formulations used in the above mentioned studies (spray or solution) in which the antibiotic is readily available for absorption, when administered as a solid form (pessaries), the active ingredient must first be released from the excipients. In cows treated with similar dosages of OTC in a spray formulation (Girardi *et al.*, 1990a), serum concentrations were lower than the detection threshold as early as 48th hour after treatment.

The prolonged persistence of the antibiotic in plasma in the present study, confirmed by both the presence of measurable levels in most of the animals (four out of five) up to 72 h after treatment and by the prolonged half-life ($t_{1/2}\beta = 21.96 \pm$ 4.42 h), may be due to the slow release of the antibiotic from the preparation used.

The prolonged persistence of OTC in milk, as reported during the present experiment (60–72 h), when compared with the times indicated by Donev *et al.* (1989) and by Girardi *et al.* (1988), 32 h and 24–48 h, respectively, can be considered as a direct consequence of plasma kinetics, which are strongly influenced by the formulation used and by the period of the cycle when the intrauterine treatment of the animals was carried out. It can be inferred from the pharmacokinetic data obtained in this study that values lower than 100 μg/kg, which have been established for OTC in milk together with its allocation in Annex I of Council Regulation (EEC) No. 2377/90, are reached after 72 h (equivalent to six milkings) following administration of uterine tablets at a dosage of 3 g/cow.

On the basis of the results obtained, it is possible to affirm that in healthy cows, following treatment with solid formulations in the early *post partum* phase, OTC is poorly and slowly absorbed from the uterus. This may also depend on the limited endoperitoneal absorption through the uterine tubes of this formulation compared to that of liquid and spray formulations (Girardi *et al.*, 1990a).

Moreover, it likely that the limited absorption of the drug from the uterus may induce a prolonged permanence of the antibiotic at high concentrations *in situ*, with the possible consequent optimization of its pharmacological effects.

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