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Submitted on 1 Jan 1992

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Slow release bolus for small ruminants: 
_in vitro_ release of tetracycline compared with serum concentrations of the antibiotic in sheep

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(Received 11 January 1991; accepted 6 March 1992)

Summary — Two formulations of slow release boluses for small ruminants containing tetracycline hydrochloride in a compressed form were investigated _in vitro_ and _in vivo_ in adult sheep. An _in vitro_ dissolution test was used for the preliminary selection of the 2 boluses. It was shown that the noncumulative kinetics of tetracycline release _in vitro_ were predictive of the tetracycline serum levels in sheep treated orally with the 2 boluses. The maximum concentrations revealed by the _in vitro_ and _in vivo_ curves were obtained at almost the same _T_m_ and, _in vivo_, the therapeutic serum levels were maintained for about 5 days. It is concluded that an _in vitro_ approach is of value in predicting the kinetic profiles of a long-acting tetracycline bolus in sheep.

slow release bolus / sheep / tetracycline

Résumé — Bolus à libération contrôlée pour les petits ruminants : comparaison de la dissolution _in vitro_ et des concentrations sériques en tétracycline chez le mouton. Deux formulations (bolus) à libération contrôlée de chlorhydrate de tétracycline ont été étudiées _in vitro_ et _in vivo_ chez le mouton adulte. Les tests de dissolution pratiqués _in vitro_ ont permis une première sélection de 2 formulations en vue de leur étude _in vivo_ chez le mouton. Il a été montré que la cinétique non cumulative de la libération de tétracycline _in vitro_ était prédictive du comportement pharmacocinétique _in vivo_ chez le mouton. Les concentrations maximales _in vitro_ et _in vivo_ ont été obtenues à des temps similaires, des concentrations ayant une valeur thérapeutique étant maintenues _in vivo_ pendant 5 j. Il est suggéré que l'approche _in vitro_ a un intérêt dans la sélection des formulations orales à longue action chez le mouton.

dispositif à libération continue / mouton / tétracycline

* Correspondence and reprints
INTRODUCTION

To satisfy the requirements of veterinary practitioners, antibiotic preparations with prolonged action and reliable therapeutic effects but which involve fewer manipulations and problems to livestock need to be developed. Although long-acting injectable oxytetracycline preparations are widely used, some of them have the disadvantage of causing local injury to tissue at the injection site (Ziv, 1980; Nouws, 1982; Xia et al, 1983; Nouws, 1984, 1990).

The aim of the present investigation was to test the suitability of the in vitro dissolution test for the preliminary selection of an appropriate slow release bolus/boluses (SRB) with tetracycline hydrochloride for small ruminants and to compare the data with those obtained in vivo.

MATERIALS AND METHODS

Drug and drug preparations

Tetracycline hydrochloride (Pharmachim, Sofia, Bulgaria) (TCH) 990 IU/mg was used as a model broad spectrum antibiotic for the development of compressed dosage preparations with prolonged action. The preparations were intended to provide effective systemic concentrations of the drug in the serum over 4–5 days and an initial peak.

The boluses were constructed on a matrix principle and contained hydrophobic and hydrophilic polymers as structure building elements. TCH was divided into “starting” and “maintaining” granules. The starting granules contained 0–10% TCH and were developed with hydrophilic polymers. The remaining quantity of the antibiotic was granulated with hydrophobic polymers in the maintaining granules. The 2 kinds of granules were compressed in bolus form. The preparation contained 6.0 g TCH. The density was fixed at 1.8 or more by means of barium sulphate granules for retention in the reticulorumen. SRB dimensions were 51/18/10 mm, and they were scored with a transversal groove. The differences between the SRB variants were based on the percentage of TCH intended for the starting dose and on the nature and the quantities of the constituent polymers.

In vitro investigations

Dissolution of the antibiotic from the preparations was investigated by means of the paddle method described in the USP XXI and modified to produce an in vitro medium as similar as possible to the physiological status of the forestomach in small ruminants. Experiments were performed using a dissolution test apparatus (Erweka-DT6, Erweka, Apparatenbau GmbH, Hausenstamm, Kr Offenbach/Main, Germany) at a temperature of 38 ± 0.5 °C (with pH 5.0–7.0 which is similar to the pH of forestomach content; Roussev et al, 1984); the eluating medium was distilled water (900 ml); more distilled water (45 ml) was added every 24 h, 30 min before sampling to compensate for the water evaporated from the cell. The paddle rotation speed was 50 rpm (Frazier and Nuessle, 1976). Distilled water was used because of the possibility of insoluble TCH chelates being formed with some cations. Samples were collected 2, 4, 6, 8, 24, 48, 72, 96 and 120 h after the beginning of the experiments. After the first 24 h one-third of the eluating medium was subsequently changed every 24 h immediately after taking a sample.

The biopharmaceutical parameters characterizing the in vitro antibiotic release from the preparations were calculated using the Weibull equation (Langenbucher, 1972, 1976) (Eq 1):

\[
M = 1 - \exp \left\{ -\left(\frac{t}{T_d}\right)^\beta \right\}
\]

[1]

In eq 1, \(M\) is the fraction of the drug dissolved at time \(t\). When there is a lag time \(t_0\), \(t = t-t_0\); \(T_d\) is a time parameter that represents the scale factor of the time axis; \(\beta\) is a shape parameter that characterizes the slope of the curve.

The dissolution efficiency (DE %) is the area under the dissolution curve of the releasing drug as a percentage of the area of the rectangle rep-
resenting 100% dissolution for the same time (Khan, 1975; Gibasier et al., 1982) was obtained from eq 2:

\[
\int_{0}^{t} 1 - \exp \left[-\left(\frac{t}{T_d}\right)^{b}\right] \, dt
\]

\[
DE\% = \frac{t}{1 - \exp \left[-\left(\frac{t}{T_d}\right)^{b}\right]} \quad [2]
\]

The area under the concentration time curve (AUC) was calculated by the trapezoidal rule. The other parameters calculated were: the rate constant \(K_a\) expressing the rate of drug release from the preparations with \(K_a = 1/T_d\), the 50% depletion time \(t_{50}\), ie the time of 50% drug release from the devices with \(t_{50} = 0.693 T_d\); the maximum concentration of the released drug \(C_{\text{max}}\) and the time to reach \(C_{\text{max}}\) \(T_{\text{max}}\) were also obtained (Langenbucher, 1972, 1976; Khan, 1975).

Mathematical calculations were performed on IBM PC using an appropriate programme. TCH release was estimated spectrophotometrically using a modified method from the State Pharmacopeia USSR X (1971).

In vivo investigations

Sixty Thracian fine-fleeced sheep (most of them male, but with some non-pregnant, non-lactating females) weighing between 40–60 kg were used. The animals were housed in boxes and were fed 0.5 kg commercial concentrate. They were given free access to hay and drinking water.

Each variant SRB was inserted orally by balling gun in 6 animals at a dose rate calculated to deliver 60–70 mg.kg\(^{-1}\)/day for 5 days, ie a total dose of 350–400 mg.kg\(^{-1}\) corresponding to 1 bolus per 15 kg body weight. Blood samples (jugular venipuncture) were collected before and after SRB administration at 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h. TCH in 10% distilled water solution was administered IV (as a single bolus injection) at a dose of 20 mg.kg\(^{-1}\) to the 6 animals. Blood samples were drawn prior to and 5, 10, 15, 30, 60, 120, 240, 360, 480, 720, 1 440 and 2 880 min after injection.

Tetracycline (TC) serum levels were determined microbiologically by agar-plate method according to Grove and Randall (1955) with Bacillus subtilis L\(_2\) as test microorganism (sensitivity 0.125 \(\mu\)g.ml\(^{-1}\)). All samples were analysed on the same day.

After IV administration of TCH, data were fitted to a biexponential equation of the form (eq 3):

\[
C_p = A \exp(-\alpha t) + B \exp(-\beta t) \quad [3]
\]

in which \(C_p\) is the TC serum concentration at time \(t\), \(A\) and \(B\) are coefficients and \(\alpha\) and \(\beta\) are exponents. Serum half-life \(t_{1/2 (\alpha)}\), volume of distribution \(V_d(\text{area})\) and body clearance \(C_{\text{fb}}\) were calculated according to Baggot (1977). AUC was estimated from zero to infinity.

The data obtained after oral administration data were fitted to equation 4:

\[
C_p = A \left[\exp(-\beta t) - \exp(-K_a t)\right] \quad [4]
\]

In eq 4, \(C_p\) is the serum concentration at time \(t\), \(A\) is a coefficient and \(K_a\) and \(\beta\) are exponents. The rate constants of the initial \((K_a)\) and of terminal \((P)\) phases, the initial half-life \((t_{1/2 (K_a)})\) and the biological half-life \((t_{1/2 (P)})\) were computed. The maximal serum concentration \(C_{\text{max}}\) and the corresponding time \(T_{\text{max}}\) were computed. The time during which the minimum inhibitory concentration of 0.5 \(\mu\)g.ml\(^{-1}\) was exceeded (Navashin and Fomina, 1982) was also calculated. The systemic bioavailability \((F)\) was computed as the ratio of the AUC after oral and IV administrations (eq 5):

\[
F = \frac{\text{AUC oral x IV dose}}{\text{AUC IV x oral dose}} \quad [5]
\]

Health monitoring (for adverse reactions) was carried out daily.

RESULTS

The IV administration of a single dose of TCH (20 mg.kg\(^{-1}\)) to sheep produced a mean serum concentration of 66.2 \(\mu\)g.ml\(^{-1}\) within 5 min of administration. The serum level then declined gradually in a biphasic manner and was detectable for up to > 24 h (fig 1). The TC half-life \((t_{1/2 (P)})\) was 6.77 h, the apparent volume of distribution
(\(V_{d(area)}\)) was 1.045 l.kg\(^{-1}\) and the body clearance (\(Cl_b\)) was 0.103 4 ml.kg\(^{-1}\).min\(^{-1}\); other parameters are given in table I.

The preliminary in vitro (and later in vivo) screening data were used to select 2 of the 30 SRB variants (Nos 68 and 69). They gave approximately the desired TCH release and the biopharmaceutical indices (table II; figs 2–4). The in vitro non cumulative concentration–time TCH curves of the selected SRB were biphasic. They showed an initial steeply rising and sharply falling phase followed by a slight rise or steady-state phase (figs 2, 3) and were accepted as suitable for SRB in small ruminants. The dissolution efficiencies of SRB 68 and 69 were similar (fig 4).

The concentration–time profiles of TC serum levels in sheep given single oral doses of SRB 68 and 69 at a rate of 1 bo-

![Graph showing concentration-time curve](image)

\[
C_p = 55.66 e^{-2.024 t} + 19.30 e^{-0.113 t}
\]

**Fig 1.** Concentration–time curve of tetracycline in the serum of sheep after IV bolus injection of a 10% aqueous solution of tetracycline hydrochloride at a rate of 20 mg.kg\(^{-1}\).

Table I. Pharmacokinetic parameters after single IV bolus injection of tetracycline hydrochloride (10% water solution) at a dose of 20 mg.kg\(^{-1}\) in 6 sheep.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Means ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) ((\mu g.ml^{-1}))</td>
<td>55.68 ± 14.12</td>
</tr>
<tr>
<td>(\alpha) (h(^{-1}))</td>
<td>2.024 ± 0.329 1</td>
</tr>
<tr>
<td>(B) ((\mu g.ml^{-1}))</td>
<td>19.30 ± 3.53</td>
</tr>
<tr>
<td>(\beta) (h(^{-1}))</td>
<td>0.113 ± 0.017 8</td>
</tr>
<tr>
<td>(t_{1/2a}) (h)</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td>(t_{1/2b}) (h)</td>
<td>6.77 ± 1.37</td>
</tr>
<tr>
<td>(AUC(0-\infty)(\mu g.h.ml^{-1}))</td>
<td>197.33 ± 22.39</td>
</tr>
<tr>
<td>(V_{d(area)}(l.kg^{-1}))</td>
<td>1.045 ± 0.219</td>
</tr>
<tr>
<td>(Cl_b(ml.kg.min^{-1}))</td>
<td>0.103 4 ± 0.011 5</td>
</tr>
</tbody>
</table>

\(A\) and \(B\): intercept terms representing the distribution and the elimination phases respectively of the serum drug concentration curve at time = 0; \(\alpha\) and \(\beta\): hybrid rate constants related to distribution and to the elimination respectively; \(t_{1/2a}\): distribution half-life; \(t_{1/2b}\) = elimination half-life; \(AUC_{(0-\infty)}\): area under serum concentration–time curve; \(V_{d(area)}\): apparent volume of distribution; \(Cl_b\): body clearance.
lus per 15 kg body weight are presented in figures 2 and 3. The pharmacokinetic parameters obtained after a single treatment of the animals with both SRB are shown in table III. The maximum TC serum concentrations determined at the 24th h in the

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### Table II. Biopharmaceutical parameters characterizing tetracycline hydrochloride release in vitro from slow release bolus (SRB) Nos 68 and 39 (mean ± SEM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>68</th>
<th>69</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE (%)</td>
<td>83.72 ± 1.92</td>
<td>81.38 ± 2.08</td>
</tr>
<tr>
<td>$K_a$ (h$^{-1}$)</td>
<td>0.047 9 ± 0.000 2</td>
<td>0.042 8 ± 0.000 2</td>
</tr>
<tr>
<td>t$_{50}$ (h)</td>
<td>12.25 ± 0.67</td>
<td>13.38 ± 0.89</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>24$^\text{a}$</td>
<td>24$^\text{a}$</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (mg.ml$^{-1}$)</td>
<td>2.568 ± 0.172$^\text{a}$</td>
<td>2.44 ± 0.158$^\text{a}$</td>
</tr>
<tr>
<td>AUC (mg.h.ml$^{-1}$)</td>
<td>548.97 ± 7.21</td>
<td>501.48 ± 4.27</td>
</tr>
</tbody>
</table>

* $^\text{a}$: Values from the non-cumulative curves; DE: dissolution efficiency; $K_a$: rate constant; t$_{50}$: 50% depletion time; $T_{\text{max}}$: time to reach maximum concentration; $C_{\text{max}}$: maximum concentration; AUC: area under concentration–time curve.

---

**Fig 2.** Concentration–time curves of tetracycline in vitro (non cumulative) (□—□) during a 5-day dissolution test and in vivo (●—●) after a single oral administration of SRB No 68 in sheep at a dose of 1 bolus per 15 kg.
Fig 3. Concentration–time curves of tetracycline in vitro (non cumulative) (□—□) during a 5-day dissolution test and in vivo (●—●) after a single oral administration of SRB No 69 in sheep at a dose of 1 bolus per 15 kg.

Table III. Pharmacokinetic parameters of slow release bolus SRB No 68 and 69 after single oral administration at a dose of 1 bolus/15 kg in 6 sheep (mean ± SEM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>68</th>
<th>SRB (No) 69</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_a$ (h$^{-1}$)</td>
<td>0.0915 ± 0.0174</td>
<td>0.0632 ± 0.0104</td>
</tr>
<tr>
<td>$\beta$ (h$^{-1}$)</td>
<td>0.0342 ± 0.0057</td>
<td>0.0264 ± 0.0026</td>
</tr>
<tr>
<td>$t_{1/2}$ $K_a$ (h)</td>
<td>8.87 ± 1.76</td>
<td>12.06 ± 1.06</td>
</tr>
<tr>
<td>$t_{1/2}$ $\beta$ (h)</td>
<td>22.48 ± 3.54</td>
<td>27.72 ± 2.09</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg.ml$^{-1}$)</td>
<td>6.66 ± 1.38$^a$</td>
<td>4.57 ± 0.69$^a$</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>19.34 ± 3.10$^a$</td>
<td>25.17 ± 2.26$^a$</td>
</tr>
<tr>
<td>$t_{e1/2}$ (h)</td>
<td>118.54 ± 12.31$^a$</td>
<td>118.98 ± 11.65$^a$</td>
</tr>
<tr>
<td>$AUC(0-\infty)$ (µg.h.ml$^{-1}$)</td>
<td>452.8 ± 40.76</td>
<td>417.5 ± 54.85</td>
</tr>
<tr>
<td>$F$ (%)</td>
<td>13.05</td>
<td>12.05</td>
</tr>
</tbody>
</table>

$^a$: Theoretical (calculated) values; $K_a$: initial rate constant; $\beta$: hybrid rate constant related to the slope of the terminal phase of the disposition curve; $t_{1/2}$ $K_a$: initial half-life; $t_{1/2}$ $\beta$: biological half-life of the terminal phase; $C_{\text{max}}$: maximum serum TC concentration; $T_{\text{max}}$: time to reach maximum concentration; $t_{e1/2}$: time to maintain TC serum above MIC (= 0.5 µg.ml$^{-1}$); $AUC(0-\infty)$: area under the concentration–time curve; $F$: systemic bioavailability.
sheep treated with SRB 68 were >6 μg. ml⁻¹. The corresponding figure (SRB No 69) was = 5 μg.ml⁻¹. The serum levels diminished gradually thereafter; on the 5th day they were within the limits of minimum inhibitory concentration (MIC), and some of them slightly below (figs 2, 3); the effective concentration (0.5 μg.ml⁻¹) appeared approximately 4 h after dosing and was maintained for more than 118 h for both SRB (table III). The terminal rate constant for SRB No 68 (0.0342 h⁻¹) and for No 69 (0.0264 h⁻¹) were significantly lower than that of β (0.1131 h⁻¹) calculated after IV TCH injection (P < 0.05). The systemic bioavailabilities of the above SRB were relatively low, i.e close to 12%.

No adverse effects (apart from one exception of slight transitory inhibition) were observed on the motor activity of the forestomach of sheep given SRB 68 and 69.

DISCUSSION

The present investigations show that the TC serum half-life obtained after IV administration in sheep corresponds to that obtained in ewe by Ziv and Sulman (1974).

The non-cumulative in vitro profile of the TCH concentration–time curves of SRB 68 and 69 may be attributed to the presence of "starting" (quick TCH release) and "maintaining" (slow TCH release) granules respectively producing the initial peak followed by the slight rise or steady state portion of the curve. This shape of the in vitro curve is a relevant one as it corresponds to
the need for SRB to provide amounts of available TCH for absorption as both loading and maintaining doses.

When the pharmacokinetic data obtained after single oral administration of both SRB and the pharmacokinetic data obtained after a single IV injection of TCH in sheep were compared, it could be seen that the biological half-lives of SRB 68 (22.48 h) and SRB 69 (27.72 h) were respectively 3 and 4 times longer than the biological half-life after TCH IV injection (6.77 h). It should also be noted that the values of the rate constants of the initial phase ($K_a$) for SRB 68 (0.0915 h$^{-1}$) and SRB 69 (0.0632 h$^{-1}$) were close to that of the rate constant of the terminal phase calculated after IV TCH dosing (0.1131 h$^{-1}$). These findings suggest that the terminal concentration–time profiles of TC after oral administration of SRB may reflect the drug absorption, associated with the rate of drug release from SRB, rather than drug elimination. In other words, it is suggested that a flip-flop effect occurs and that the half-life of TC elimination calculated after oral administration of SRB corresponds to an absorption half-life (Curry, 1980; Ritschel, 1980). The likely explanation for a prolonged dissolution process is the composition of the SRB 68 and 69, which consists of a rapidly soluble portion and a slower dissolving portion.

The low bioavailability (= 12–13%) detected in the present experiment could be connected with the structure of the devices (lying at the bottom of the reticulorumen, relatively slow disintegration in granules and their subsequent mixing and dilution in the large content of the forestomach) and with the influence of the gastrointestinal environment on the antibiotic (partial fixing on the feed particles, chelate building and disintegration). Nevertheless, the serum concentrations obtained show that SRB 68 and SRB 69 are able to ensure prolonged effective serum TC levels in sheep. Of particular interest is the finding that the duration of the TC serum concentration is in agreement with results established from in vitro studies. The data of selected SRB on the noncumulative kinetics of TCH release in vitro compared with the in vivo TC serum levels in sheep showed that the initial peaks of TCH in vitro and in vivo of each SRB coincide TC equivalent (with respect to $C_{max}$ and $T_{max}$). Some parallelism was also established between other biopharmaceutical in vitro parameters and TC pharmacokinetic parameters after single oral administration of both SRB in sheep, especially for the times of 50% TC release in vitro ($t_{50}$) and the apparent TC absorption half-lives.

In conclusion, an in vitro dissolution test could help in selection of a suitable TCH SRB preparation of compressed type for use in sheep. Such an approach should be encouraged to avoid wasteful in vivo experiments with inappropriate preparations.

REFERENCES


Langenbucher F (1972) Linearization of dissolution rate curves by the Weibull distribution. *J Pharm Pharmacol* 24, 979-981

Langenbucher F (1976) Parametric representation of dissolution rate curves by the RRSBW distribution. *Pharm Ind* 38, 472-477


Nouws JFM (1984) Irritation, bioavailability and residue aspects of ten oxytetracycline formulations administered intramuscularly to pigs. *Vet Q* 6, 80-84


