

Pharmacokinetics and tissue residues of an oxytetracycline/diclofenac combination in cattle

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Summary

Eight male cattle were given a combined dose containing 20 mg/kg oxytetracycline and 0.5 mg/kg diclofenac intramuscularly. Blood samples were drawn at different times until 168 h after administration. Two experimental animals were slaughtered by humane means at weekly intervals up to 28 days after administration. Samples of muscle, injection zone tissue, liver, kidney and fat were obtained.

Oxytetracycline and diclofenac concentrations were determined by high performance liquid chromatography. Kinetic analysis was performed by linear regression using the CSTRIP programme.

Plasma oxytetracycline concentration showed a maximum (C_{max}) of 3.89 ± 1.48 $\mu\text{g/ml}$ and a prolonged elimination half-life ($T_{1/2\beta}$: 47.73 ± 18.33 h). The diclofenac plasma profile showed high C_{max} (577.62 ± 238.40 ng/ml), and its $T_{1/2\beta}$ was also prolonged (30.48 ± 9.42 h). Oxytetracycline concentrations were measurable in liver and adipose tissue until day 21 after administration, but all tissue samples were negative for diclofenac at 21 days. The long elimination half-life of diclofenac was an unexpected finding; its $T_{1/2\beta}$ in humans is 1.1 h.

Keywords

Antimicrobial – Cattle – Diclofenac – Non-steroidal anti-inflammatory – Oxytetracycline – Pharmacokinetics – Residue.

Introduction

The combination of antimicrobials and anti-inflammatory agents in therapeutics has long been considered advantageous in certain situations in human and veterinary medicine. There are few data available, however, on the pharmacokinetics and tissue residues of these drugs when applied simultaneously.

Oxytetracycline (OTC) is a broad-spectrum antimicrobial agent that is active against bacteria and also against some

chlamydiae, rickettsiae and protozoa. It is widely used in veterinary medicine because of its wide spectrum and advantageous pharmacokinetic features (5, 33, 39).

The popularity of OTC in Latin America for treating cattle diseases prompted a review of the clinical efficacy and pharmacokinetics of OTC (29) and earlier reviews of the pharmacology of tetracyclines in general have been published (23, 25). Numerous reports have described the pharmacokinetics of OTC, including those that describe intravenous (IV) pharmacokinetic analysis using bi- and tri-compartmental models (31, 32, 45, 49).

Long-acting formulations allow inhibitory concentrations of OTC to persist for longer periods than conventional preparations, potentially producing improvements in efficacy and longer persistence of residues.

A previous report has examined the tissue residue profile of a long-acting OTC preparation (24). However, the introduction of different long-acting OTC formulations has created the need for additional bioequivalence studies (28).

Non-steroidal anti-inflammatory drugs (NSAIDs) are often combined with antimicrobial agents to treat systemic infections accompanied by fever and other inflammatory conditions (11).

Pharmacokinetic interactions between antibiotics and NSAIDs have been described in experimental animal models (9, 10, 20). To the best of the authors' knowledge, the work described here is the first kinetic study of simultaneous administration of the NSAID diclofenac and the antibiotic oxytetracycline. Non-steroidal anti-inflammatory drugs may improve the efficacy of antibiotics through pharmacokinetic interactions, as demonstrated in the experimental studies of Joly *et al.* (20). In human cases of chronic osteoblastic osteomyelitis, some investigators have proposed prescription of an NSAID with the antibiotic treatment (44). It is therefore important to assess the interactions between these two drug families.

Diclofenac (DCF) is a potent NSAID of the phenylacetic acid class, which has good analgesic, antipyretic and anti-inflammatory properties, but has had only limited use in veterinary medicine (2, 21). Diclofenac sodium is extensively used in the treatment of animals in India, Pakistan and Bangladesh (38). As a consequence, the presence of residues of the drug in domestic animal products is a common finding. Recently, the toxic effects of meat of domestic animals treated with DCF were reported in three vulture species in India and Pakistan. Intoxicated vultures died probably as a consequence of DCF-induced visceral gout (36). High levels of DCF residues found in the tissues of dead vultures were attributed to scavenging of the carcasses of domestic animals that had previously been inoculated with DCF. The authors of the study concluded that DCF may have been involved in the death of the vultures and the consequent decline in their population. In recent studies, DCF residues and renal disease (visceral gout) have been experimentally reproduced in Oriental white-backed vultures fed with meat obtained from DCF-treated buffaloes (34). A further toxicity study was carried out by Prakash Reddy *et al.* (38) in DCF-treated poultry, in which neither major biochemical nor histopathological changes were observed 28 days after administration. There is still some controversy regarding the mode of use and dosing of DCF by paraveterinarians and farmers in India, and the true toxicokinetics and dynamics of DCF in vultures need to be clarified.

Diclofenac irreversibly inhibits the cyclooxygenase pathway of prostaglandin synthesis, which is the most common mediator of pain, inflammation and pyrexia (7). In humans, DCF has a short half-life of elimination (47) and is rapidly and completely absorbed after oral administration. Peak concentrations in plasma are reached in 2 h to 3 h; it is extensively bound to plasma proteins (99%), is metabolised in the liver and its metabolites are excreted mainly in urine (26).

The fact that DCF is widely used in human medicine for the long-term symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis suggests that the residue levels found in animal products are safe for humans, especially considering that these residue levels are far below the therapeutic levels given to human patients.

The objectives of the present paper were to determine the pharmacokinetic behaviour of OTC and DCF administered in combination to cattle, and to determine the DCF and OTC residual profiles in edible tissues.

Materials and methods

Experimental animals

The study was conducted in eight healthy, young, castrated male Aberdeen Angus cattle weighing 170 kg to 230 kg. The animals were housed and fed an antibiotic-free diet (alfalfa hay and straw) for two months before and during the study period and water was available *ad libitum*. The experimental animals received 20 mg/kg OTC combined with 0.5 mg/kg DCF intramuscularly in the neck. The commercial formulation used contains 20% OTC and 0.5% DCF (Terravet® L.A. Plus, Intervet, Argentina). Blood samples were obtained immediately before injection and at the following times post-administration: 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 48, 72, 96, 120, 144 and 168 h. Samples were obtained into heparin tubes, and the plasma was separated and stored at -20°C. Two experimental animals were slaughtered, using a pneumatic stunner followed by exsanguination, on days 7, 14, 21 and 28 post-administration. Samples of gluteal muscle, injection zone tissue, liver, kidney and adipose tissues were obtained from each animal.

Oxytetracycline and diclofenac assays

Chemicals

Pure reference standards of oxytetracycline (Sigma, USA) and diclofenac (Intervet, Argentina) were used for the validation of the analytical methodology. All the solvents and reagents used during the extraction and drug analysis were high pressure liquid chromatography (HPLC) grade

and purchased from J.T. Baker Inc. (Phillipsburg, USA). Water was double distilled and deionised using a water purification system (Simplicity®, Millipore, Brazil).

Analysis of oxytetracycline

Sample pre-treatment: plasma

Oxytetracycline plasma concentrations were determined by HPLC with ultraviolet (UV) detection after solid phase extraction with C₁₈ cartridges (J.T. Baker, Inc.) by means of a modification of the method reported by Oka (35). To 1 ml of plasma was added 4 ml of ethylenediamine tetraacetic acid (EDTA) McIlvaine buffer (4°C); the mixture was centrifuged at 3,600 × g for 10 min, and the supernatant was cleaned with solid-phase extraction (SPE) using C₁₈ cartridges. The EDTA-McIlvaine buffer was prepared as follows: dibasic sodium phosphate (17.76 g) and citric acid monohydrate (21.01 g) were dissolved in 625 ml and 1000 ml of distilled water, respectively. These solutions were mixed together and then disodium EDTA (60.49 g) was added. The pH of the solution was adjusted to 4.0 ± 0.1 using orthophosphoric acid.

Sample pre-treatment: tissue

The method used was a modification of the technique reported by Walsh *et al.* (46). Briefly, 2 g of the tissue sample was homogenised with 5 ml of EDTA-McIlvaine buffer pH 4 (4°C) using an Ultraturrax homogeniser (IKA Works Inc., USA). The homogenate was centrifuged at 3,600 × g for 15 min, and the supernatant was collected. A volume of trichloroacetic acid solution (1 g/ml) equal to 10% of the supernatant volume was added slowly to the supernatant, with constant stirring. The mixture was stirred for a further 1 min, then placed in a bed of ice for 15 min, filtered and extracted by the solid-liquid method with C₁₈ cartridges (Baker, Inc.).

For both types of sample (plasma and tissue), the C₁₈ cartridge was conditioned with 5 ml of methanol and then with 5 ml of EDTA-McIlvaine buffer. The outlet of the SPE cartridge was connected to a water pump, and the sample was aspirated through the cartridge at no more than 10 ml/min. When the sample had passed through, the SPE cartridge was disconnected and flushed with 5 ml of EDTA-McIlvaine buffer. The elution was performed with 2 ml of a 50:50 mixture of acetonitrile and EDTA-McIlvaine buffer. A 200 µl volume of this eluate was injected into the liquid chromatograph.

High pressure liquid chromatography

The HPLC system consisted of an isocratic pump (Gilson Inc., France, Model 307) with flow rate set at 2 ml/min, a 5 µm LiChrocart® 125-4 RP₁₈ analytical column (Merck KGaA, Germany) in series with a 5 µm C₁₈ Guard-Column,

and a UV-VIS detector (Gilson Inc. Model 155) set at a wavelength of 365 nm. The chromatographic separation was performed in reversed-phase mode using a mobile phase of 72% 0.01 M citric acid–0.01 M dipotassiumorthophosphate and 28% acetonitrile, containing 0.005 M tetramethylammonium chloride and 0.1 g/l EDTA.

Analysis of diclofenac

Sample pre-treatment: plasma

Diclofenac plasma concentrations were determined by HPLC after liquid–liquid extraction (8). Four millilitres of 2.5 M phosphoric acid was added to a 1 ml sample of plasma. After the sample had been vortexed for 10 s, 5 ml hexane:isopropyl alcohol (90:10) was added. The sample was shaken for 15 min, and then centrifuged for 15 min at 3,600 × g. The organic phase was transferred to a culture tube using an automated pipette. This process was repeated once again, and then the combined organic phases were evaporated under a stream of nitrogen at 45°C. The residue was reconstituted in 100 µl of mobile phase solution. The sample was vortexed for 1 min and then a 50 µl aliquot was injected into the chromatographic system.

Sample pre-treatment: tissue

Diclofenac tissue concentrations were determined by HPLC after liquid–liquid extraction. The samples (2 g) were homogenised with 4 ml of 2.5 M phosphoric acid, and DCF was extracted three times with 5 ml hexane:isopropyl alcohol (90:10). The combined organic phases were evaporated under a stream of nitrogen at 45°C. The residue was reconstituted in 100 µl of mobile phase solution. The sample was vortexed for 1 min and then injected into the chromatographic system.

High pressure liquid chromatography

The HPLC system consisted of an isocratic pump (Gilson Inc. Model 307) with flow rate set at 1.5 µl/min, a 5 µm LiChrocart® 125-4 RP₁₈ analytical column (Merck KGaA, Germany) in series with a 5 µm C₁₈ Guard-Column, and a UV-VIS detector (Gilson Inc. Model 155) set at a wavelength of 280 nm. The chromatographic separation was performed in reversed-phase mode using a mobile phase of 25% methanol, 20% acetonitrile and 55% 0.08 M phosphate buffer (v/v), pH 7.0 ± 0.1, at room temperature.

Calibration and validation

The assays were validated by measuring the concentrations of known amounts of OTC and DCF in cattle plasma and tissue. The linearity, precision, accuracy and specificity were calculated for each compound (n = 6 samples of

each). The absolute recovery of each compound was assessed at different concentrations (between 0.062 and 1 µg/ml or µg/g for OTC, and between 6.25 and 100 ng/ml or µg/g for DCF) by quintuple analysis. The extraction efficiency for the two molecules under study was determined by comparison of the detector responses obtained for the peak areas of fortified blank samples with the peak areas resulting from direct injection of equivalent quantities of standard solutions.

Linearity of standard curves

The ratio between different concentrations of the drug under study and chromatographic peak areas was determined for OTC and DCF. Calibration curves were obtained for OTC (concentrations ranging between 0.062 and 1 µg/ml) and for DCF (concentrations ranging between 6.25 and 100 ng/ml).

Precision and accuracy

The between-day precision was determined to estimate the run-to-run variation in the extraction and chromatographic methods. Between-day variation was measured on five consecutive working days for plasma and tissue OTC and DCF samples. Precision was expressed as the coefficient of variation (% CV) (42).

Accuracy is defined as the agreement between the experimentally measured and the true value. Accuracy of the method was assessed by the differences between observed and calculated concentration results obtained within-day and between-day (on five consecutive working days), and was expressed as the relative error (% RE) (42).

Limit of quantitation

The limit of quantitation (LOQ) was calculated ($n = 6$) as the lowest OTC or DCF concentration on the standard curve that could be quantitated with precision (CV not exceeding 15%) and an accuracy within 20% of the nominal (42).

Pharmacokinetic analysis

The pharmacokinetic models that best fitted the OTC and DCF plasma profiles were determined by means of the minimum Akaike information criterion estimation (MAICE) test (48), which uses the Akaike information criterion (AIC) (1), defined by:

$$AIC = N \ln Re + 2p.$$

Where N is the number of experimental data points, p is the number of parameters in the estimated model, and Re is the residual sum of squares.

Plasma OTC and DCF concentrations at the various time points were tabulated and the means and standard deviations calculated. The pharmacokinetic estimates were obtained by least squares linear regression (LSLR) analysis using the CSTRIP program (40). The peak OTC and DCF concentrations (C_{max}) and peak time (T_{max}) for each animal were read from the concentration-time curve. The absorption rate ($T_{1/2abs}$), the concentration-time intercept (C_0), the area under the curve (AUC) obtained using the trapezoidal rule, and the plasma elimination half-lives of OTC and DCF ($T_{1/2\beta}$) were calculated.

The area under the plasma concentration-time curve, $AUC_{(last)}$, from time zero to the latest time (t) with a measurable concentration (C_p) was estimated using linear trapezoidal approximation. The area from time t to infinity was estimated as C_p/β , where β represents the terminal elimination rate constant. The total area under the curve was estimated as the sum of the two, i.e.

$$AUC_{(0-\infty)} = AUC_{(last)} + AUC_{(t-\infty)}.$$

Beta was estimated using least squares regression analysis of the log concentration-time data obtained during the terminal log-linear elimination phase. The elimination half-life ($T_{1/2}$) was calculated as $0.693/\beta$.

Statistical moment theory was applied to calculate the mean residence time (MRT) for each compound as follows (37):

$$MRT = (AUMC / AUC) - (1 / K_{abs}).$$

Where AUMC is the area under the curve of the product of time and drug concentration vs time from zero to infinity (18) and K_{abs} represents the first order absorption rate constant. The AUMC was used to estimate the volume of distribution at steady-state ($Vd_{(ss)}$) according to the following equation:

$$Vd_{ss} = (Dose / AUC) \times (AUMC / AUC).$$

Total body clearance (Cl_B) was calculated using:

$$Cl_B = Dose / AUC.$$

Because the intravenous (IV) route was not used, the $Vd_{(ss)}$ and Cl_B represents the $Vd_{(ss)}/F$ or Cl_B/F . Bioavailability (F) values were not estimated. The Vd and Cl_B values are expressed as referring to systemic bioavailability (see Tables I and II).

Table I
Individual and mean ± standard deviation (SD) oxytetracycline plasma pharmacokinetic parameters after intramuscular administration of an oxytetracycline-diclofenac combination to cattle at 20 mg/kg

Parameter	1	2	3	4	5	6	7	8	Mean	SD
K_{abs} (h ⁻¹)	1.16	1.67	1.51	3.86	2.97	1.73	0.55	0.78	1.78	1.12
$T_{1/2abs}$ (h)	0.60	0.42	0.46	0.18	0.23	0.40	1.26	0.89	0.56	0.36
C_{max} (µg/ml)	2.65	2.48	3.65	3.66	2.92	6.93	5.07	3.73	3.89	1.48
T_{max} (h)	4	2	2	2	2	4	4	4	3.00	1.07
α (h ⁻¹)	1.09	0.18	0.40	0.39	1.42	1.64	0.28	0.44	0.73	0.57
$T_{1/2\alpha}$ (h)	0.64	3.84	1.72	1.78	0.49	0.42	2.48	1.58	1.62	1.16
B (µg/ml)	1.87	1.61	2.20	1.73	2.27	3.45	2.38	2.31	2.23	0.57
β (h ⁻¹)	0.018	0.024	0.031	0.0098	0.013	0.016	0.0095	0.014	0.017	0.01
$T_{1/2\beta}$ (h)	38.82	28.43	22.34	70.61	55.66	43.46	73.19	49.36	47.73	18.33
AUC_{∞} (µg.h/ml)	108.68	69.20	78.41	182.95	187.70	232.55	258.50	173.32	161.41	69.65
AUMC (µg.h ² /ml)	5,914.17	2,704.65	2,265.50	18,041.55	14,907.24	13,754.85	26,573.05	11,780.19	11,992.65	8,269.18
MRT (h)	52.42	37.08	29.89	96.61	77.42	57.15	100.80	65.97	64.67	25.82
$Vd_{(ss)}/F$ (l/kg)	8.68	9.65	6.17	9.51	7.42	4.42	7.02	6.85	7.46	1.77
Cl_B/F (ml/h/kg)	165.63	260.12	229.57	98.39	95.90	77.40	69.63	103.85	137.56	72.62

K_{abs} : slope of the absorption phase of the IM drug-disposition curve
 $T_{1/2abs}$: absorption half-life (0.693/ K_{abs})
 C_{max} : maximum observed serum concentration
 T_{max} : time at which the maximum concentration occurred
 α : slope of the distribution phase of the IM drug-disposition curve
 $T_{1/2\alpha}$: distribution half-life (0.693/ α)
B : zero-time intercept of the elimination phase of the IM drug-disposition curve
 β : slope of the elimination phase of the IM drug-disposition curve
 $T_{1/2\beta}$: elimination half-life (0.693/ β)
AUC : area under the plasma OTC concentration-time curve
MRT : mean residence time
 $Vd_{(ss)}/F$: apparent volume of drug distribution at steady state
 Cl_B/F : total body clearance
Vd - Cl : represent their true values divided by the systemic availability (F)

Table II
Individual and mean ± standard deviation (SD) diclofenac plasma pharmacokinetic parameters after intramuscular administration of an oxytetracycline-diclofenac combination to cattle at 0.5 mg/kg

Parameter	1	2	3	4	5	6	7	8	Mean	SD
K_{abs} (h ⁻¹)	0.31	0.268	0.697	0.83	0.39	0.46	0.31	0.57	0.48	0.20
$T_{1/2abs}$ (h)	2.24	2.59	0.995	0.83	1.77	1.50	2.20	1.22	1.67	0.64
C_{max} (ng/ml)	555.54	367.45	633.30	419.28	825.57	194.76	869.95	755.09	577.618	238.40
T_{max} (h)	10	10	10	8	8	6	12	8	9.00	1.85
α (h ⁻¹)	0.25	0.024	0.69	0.82	0.31	0.12	0.27	0.52	0.37	0.28
$T_{1/2\alpha}$ (h)	2.75	29.38	1.02	0.85	2.21	5.91	2.55	1.34	5.75	9.68
B (ng/ml)	65.54	446.11	320.59	141.08	124.46	54.28	87.05	140.98	172.51	138.26
β (h ⁻¹)	0.016	0.039	0.038	0.025	0.025	0.017	0.0195	0.0199	0.025	0.01
$T_{1/2\beta}$ (h)	42.74	17.70	18.05	27.53	27.47	40.04	35.48	34.80	30.48	9.42
AUC_{∞} (ng.h/ml)	8,895.96	11,824.3	10,909	6,657.77	9,354.54	4,525.14	13,692.3	9,857.31	9,464.54	2,885.78
AUMC (ng.h ² /ml)	296,771.3	315,018.4	242,099.1	240,845.9	231,440.2	191,226.7	324,518.1	371,137	276,632.09	59,657.25
MRT (h)	30.027	23.3083	18.8593	32.8418	21.4076	38.9254	20.3675	34.3176	27.507	7.48
$Vd_{(ss)}/F$ (l/kg)	1.52	0.89	0.78	2.22	1.03	3.87	0.67	1.57	1.568	1.06
Cl_B/F (ml/h/kg)	50.58	38.06	41.25	67.59	48.11	99.44	32.87	78.52	57.05	22.95

K_{abs} : slope of the absorption phase of the IM drug-disposition curve
 $T_{1/2abs}$: absorption half-life (0.693/ K_{abs})
 C_{max} : maximum observed serum concentration
 T_{max} : time at which the maximum concentration occurred
 α : slope of the distribution phase of the IM drug-disposition curve
 $T_{1/2\alpha}$: distribution half-life (0.693/ α)
B : zero-time intercept of the elimination phase of the IM drug-disposition curve
 β : slope of the elimination phase of the IM drug-disposition curve
 $T_{1/2\beta}$: elimination half-life (0.693/ β)
AUC : area under the plasma OTC concentration-time curve
MRT : mean residence time
 $Vd_{(ss)}/F$: apparent volume of drug distribution at steady state
 Cl_B/F : total body clearance
Vd - Cl : represent their true values divided by the systemic availability (F)

Results

Validation of the analytical procedure

The plasma OTC limit of quantitation was 0.062 µg/ml, and an average correlation coefficient (r) of 0.9925 was obtained, indicating a good fit to the least-squares weighted linear regression model. The mean plasma recovery was 85% and the recovery from fortified tissue samples was 82%, 80%, 78% and 76% for fat, liver, kidney and muscle, respectively. The LOQ was 0.06 µg/g for all tissues. The inter-assay precision (relative standard deviation) was <5%, 10%, 7%, 8% and 7% for plasma, fat, liver, kidney and muscle, respectively.

The plasma and tissue DCF LOQ was 6.25 ng/ml and 6.25 ng/g, respectively; an average correlation coefficient (r) of 0.9982 was obtained, indicating a good fit to the least-squares weighted linear regression model. The mean slope data were associated with a coefficient of variation (CV) of 7.30%, indicating good between-day reproducibility. The mean plasma recovery was 87% and the recovery from fortified tissue samples was 78%, 80%, 75% and 83% for fat, liver, kidney and muscle, respectively. The between-day coefficient of variation was 5.3% to 13.3% for the entire concentration range (6.25 to 100 ng/ml or ng/g).

Pharmacokinetics

The mean OTC levels found in plasma after the intramuscular (IM) administration of combination OTC-DCF to bovines are depicted graphically in Figure 1, and Table I presents the IM pharmacokinetic data for OTC.

Oxytetracycline plasma concentrations evolved following known features for 20% formulations: high C_{max} (3.89 ± 1.48 µg/ml) and prolonged half-life of elimination (47.73 ± 18.33 h).

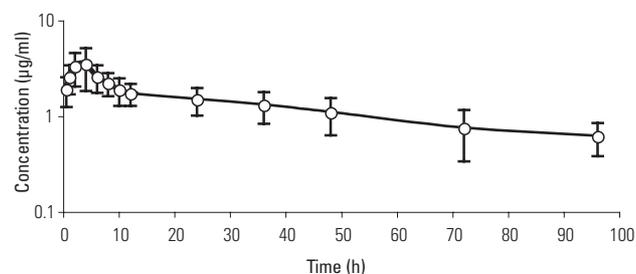


Fig. 1
Oxytetracycline plasma concentrations (mean \pm standard deviation) after intramuscular administration of an oxytetracycline-diclofenac combination to cattle. The bars represent the standard deviation of the experimental data

The mean DCF levels found in plasma after the IM administration of combined OTC-DCF to cattle are depicted graphically in Figure 2, and Table II presents the IM pharmacokinetic data for DCF.

The DCF plasma profile was very interesting. As with OTC, its pharmacokinetic characteristics were a high C_{max} (577.62 ± 238.40 ng/ml) and prolonged plasma persistence ($T_{1/2\beta} = 30.48 \pm 9.42$ h).

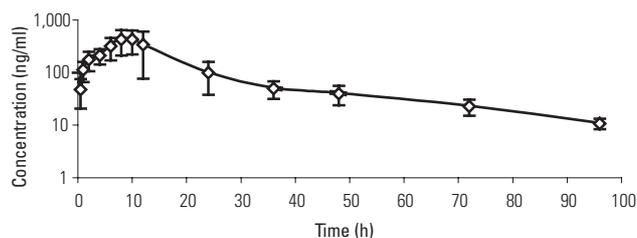


Fig. 2
Diclofenac plasma concentrations (mean \pm standard deviation) after intramuscular administration of an oxytetracycline-diclofenac combination to cattle. The bars represent the standard deviation of the experimental data

Oxytetracycline is a lipophilic drug with a high volume of distribution (V_d) and, as a consequence, high tissue concentrations are obtained. Tissue concentrations of OTC after IM injection of the combined OTC-DCF were measured for up to 28 days in liver, with a mean value of 0.12 µg/g, up to 21 days in fat and injection zone tissue, with mean values of 0.20 and 0.82 µg/g, and up to 14 days in kidney and muscle, with mean values of 0.47 and 0.27 µg/g respectively (Fig. 3 and Table III).

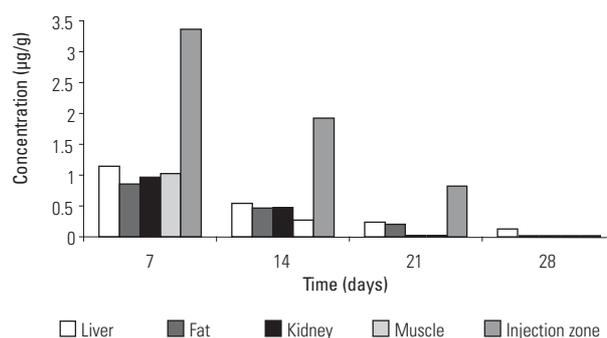


Fig. 3
Oxytetracycline tissue concentrations (µg/g) after intramuscular administration of an oxytetracycline-diclofenac combination to cattle

Diclofenac tissue concentrations after IM injection of the combined OTC-DCF were measured for up to 14 days in liver, fat, kidney, muscle and injection zone tissue, with mean values of 39.11, 18.73, 29.26, 15.8 and 46.51 ng/g, respectively (Fig. 4 and Table IV).

Table III
Mean oxytetracycline tissue concentrations (µg/g) after intramuscular administration of an oxytetracycline-diclofenac combination to cattle (n = 2 samples per site)

Tissue	7 days	14 days	21 days	28 days
Liver	1.14	0.54	0.23	0.12
Fat	0.85	0.46	0.2	nd
Kidney	0.96	0.47	nd	nd
Muscle	1.02	0.27	nd	nd
Injection zone	3.36	1.92	0.82	nd

nd : not detected

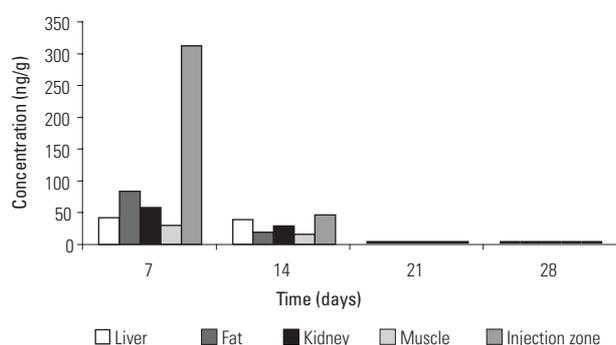


Fig. 4
Diclofenac tissue concentrations (ng/g) after intramuscular administration of an oxytetracycline-diclofenac combination to cattle

Table IV
Mean diclofenac tissue concentrations (ng/g) after intramuscular administration of an oxytetracycline-diclofenac combination to cattle (n = 2 samples per site)

Tissue	7 days	14 days	21 days	28 days
Liver	42.42	39.11	nd	nd
Fat	83.27	18.73	nd	nd
Kidney	57.56	29.26	nd	nd
Muscle	29.6	15.8	nd	nd
Injection zone	312.18	46.51	nd	nd

nd : not detected

Discussion

When bacteriostatic drugs such as OTC are administered, the serum drug levels should not decrease below the effective concentrations at any time during the period of treatment (45). Because repeated dosage of OTC is not always practical, long-acting formulations have been developed to achieve high blood levels rapidly and to provide a greater effective serum concentration over several days (45).

In any pharmacokinetic study the selection of the calculation method is a key issue. The selection of mono-, bi- or tri-exponential equations to calculate compartmental parameters could result in significant differences in the estimated parameters; in this regard the correct selection of the weighting procedure is the key to obtaining the best estimates (14).

In the present study, the plasma OTC profile could be described by a two-compartment open model with first-order absorption. In experiments on cows and ewes, Ziv and Sulman (49) found that the elimination of OTC was best described by the two-compartment open model, and this model was also used by Bretzlaff *et al.* (6) in their experiments on cows. This is in agreement with other studies in various animal species (27, 43, 45).

Absorption of OTC from the injection site after IM injection resulted in plasma concentrations of 2.58 ± 0.66 µg/ml within 60 min, with a maximum of 3.89 ± 1.48 µg/ml obtained 3 h after administration of the DCF-OTC combination. Plasma concentrations then decreased slowly, but exceeded 0.5 µg/ml for 96 h after injection. The elimination half-life was long (47.73 ± 18.33 h); this value is coincident with that reported by the present authors in a pharmacokinetic study of OTC administered alone at the same dose to calves (34.98 ± 15.88) (28), and is also in agreement with that found by Landoni (37.82 h) (23). This appears to be a logical value and occurs because of the slow release formulation. In experiments in calves, Nouws *et al.* (31) reported a slight retard-effect of long-acting OTC; this effect depends on the tissue damage produced at the injection site. Nevertheless, Toutain and Raynaud (45) reported an apparent half-life of elimination of 26.2 h. The differences in the half-life of OTC reported by different authors, which have been attributed to differences in formulation, dose, route of administration, age and diet, might also be related, at least partially, to the use of linear or nonlinear regression analysis and, in the latter case, to the use of different weighting procedures in the calculations (14).

The area under the OTC curve was 161.41 µg.h/ml, this is coincident with the value reported in calves that received the same dose of OTC alone (168 ± 14.6 µg.h/ml) (12), and with the area under the curve calculated by Landoni after the administration of the same dose by the same route to female Hereford calves (174.29 µg.h/ml) (23). The $V_{d(ss)}$ was high (7.46 l/kg); this is in agreement with Escudero *et al.* (15), who reported that a 20 mg/kg dose of a long-acting OTC formulation administered to sheep gave mean values of $AUC = 112$ µg/ml and $V_{d(area)} = 7.01$ l/kg, which indicates that OTC is well distributed in the body.

In 1998 the Food and Drug Administration of the USA established new tissue tolerances for the tetracycline family

of drugs based on their effect on intestinal microflora (4, 17). These tolerances represent the permitted sum of tetracycline residues, to include tetracycline, chlortetracycline and OTC. Cattle tolerances are 2 ppm for muscle, 6 ppm for liver, and 12 ppm for both kidney and fat. The data produced in this study show that cattle tissues are below the cattle tolerance for OTC 28 days after dosing with OTC-DCF.

The calculated value of the elimination half-life for DCF ($T_{1/2\beta} = 30.47 \pm 9.42$ h) was very high compared with the 1.1 h reported after IV administration (47) and the 1.15 h after IM injection (22) in humans, which shows that the drug is removed at a slower rate from calves than humans. The value found in the present study was higher than the elimination half-life reported for buffalo calves ($T_{1/2\beta} = 12.84 \pm 1.29$ h) following IV administration of DCF (1mg/kg) with enrofloxacin (4 mg/kg) in two different syringes one immediately after the other (21). The value reported here was higher also than the elimination half-life obtained in a bioavailability study performed in lactating cows that received 2.5 mg DCF/kg IM (11.3 h) (16), and ten times higher than elimination half-lives obtained in sheep following IV and IM administration of DCF alone (2 h to 3 h for both routes of administration) (2). One explanation for this finding could be a possible influence of the OTC formulation on the pharmacokinetic behaviour of DCF. This is a hypothesis only, because no study of DCF alone has been carried out. This profile agrees with a 'flip flop' kinetic behaviour, in which the rate of absorption dominates the terminal phase of the plasma concentration-time curve.

The value of the volume of distribution of DCF reported in humans (0.17 ± 0.11 l/kg) (47) is significantly lower than the value of 1.57 ± 1.06 l/kg observed in the present study. In the present study, high tissue levels of DCF were found at 7 and 14 days after administration, which correlates with a high Vd. The Vd value is in agreement with that reported by Kumar *et al.* (21) in buffalo calves after concomitant administration of DCF and enrofloxacin. Although this parameter generally indicates that there may be extensive penetration of DCF into various body fluids, it should be noted that slow and probably incomplete absorption from the injection site also occurs, which correlates strongly with the high concentrations found in the injection site samples 7 and even 14 days after administration. This phenomenon probably contributes to the high Vd/F values.

On 17 September 2003 the Committee for Veterinary Medicinal Products of the European Medicines Agency recommended the establishment of tissue Maximum Residue Limits (MRLs) for DCF in bovine animals in accordance with Council Regulation (EEC) No. 2377/90. The cattle MRLs are 5 ng/g for muscle and liver, 10 ng/g for kidney and 1 ng/g for fat (16). The MRL for adipose tissue is smaller than the limit of quantitation used here, but the data produced in this study show that cattle tissues are below the cattle MRL for DCF 21 days after dosing with OTC-DCF.

Shepherd *et al.* (41) studied the comparative efficacy and safety of a diclofenac-gentamicin combination and gentamicin alone in eye drops. They concluded that the combination was more effective than gentamicin alone in the control of inflammation following cataract surgery, and appeared to be as safe. The anti-inflammatory agent diclofenac sodium has also exhibited remarkable antibacterial effects both *in vitro* and *in vivo* (3); synergism between DCF and streptomycin has been demonstrated to be statistically significant compared with the individual effects of the drugs. Diclofenac also has demonstrable antimicrobial activity against pathological bacterial species (16).

In another study, Nergelius *et al.* (30) reported that DCF does not alter cloxacillin pharmacokinetics, and neither cloxacillin nor DCF in single IV doses causes renal dysfunction. In contrast, Groppo *et al.* (19) demonstrated that sodium diclofenac significantly reduced serum and tissue amoxicillin concentrations.

Doherty *et al.* (13) have demonstrated the efficacy of OTC and flunixin meglumine combination therapy in calves suffering from pneumonia. Availability of a treatment suitable for administration by a single injection that combines a long-acting antibiotic and an anti-inflammatory agent offers advantages of therapeutic efficacy and practical convenience.

In this study, high and persistent OTC levels in plasma and tissues were found in treated cattle. In conclusion, the pharmacokinetics of OTC appear not to be influenced by the simultaneous administration of DCF at a dose rate of 0.5 mg/kg. Further efficacy studies are planned to corroborate these results.



Pharmacocinétique et taux tissulaire de résidus suite à l'administration d'une association d'oxytétracycline et de diclofénac à des bovins

N. Mestorino, E. Mariño Hernández, L. Marchetti & J.O. Errecalde

Résumé

Huit bœufs adultes ont reçu par voie intramusculaire une dose contenant une combinaison de 20,0 mg/kg d'oxytétracycline et de 0,5 mg/kg de diclofénac. Des prises de sang ont été effectuées à différents intervalles jusqu'à 168 heures post-administration. Deux animaux expérimentaux ont été sacrifiés de manière humaine à une semaine d'intervalle durant les quatre semaines suivant l'administration. Des échantillons du muscle, des tissus de la zone d'injection, du foie, des reins et de la masse grasseuse ont été prélevés.

Les concentrations d'oxytétracycline et de diclofénac ont été mises en évidence par chromatographie en phase liquide de haute performance (HPLC). L'analyse cinétique a été effectuée par régression linéaire en utilisant le programme CSTRIP.

Le niveau maximum de concentration (C_{max}) d'oxytétracycline dans le plasma était de $3,89 \pm 1,48$ µg/ml avec une demi-vie d'élimination prolongée ($T_{1/2\beta}$: $47,73 \pm 18,33$ h). La C_{max} de diclofénac était d'un niveau élevé ($577,62 \pm 238,40$ ng/ml), avec une demi-vie d'élimination également prolongée ($30,48 \pm 9,42$ h). Des concentrations d'oxytétracycline ont pu être mesurées dans le foie et les tissus adipeux jusqu'au 21^e jour post-administration, tandis que le diclofénac avait disparu de tous les échantillons de tissus au 21^e jour. La longueur de la demi-vie d'élimination du diclofénac a été une observation inattendue ; chez l'homme, elle est de 1,1 h.

Mots-clés

Anti-inflammatoire non stéroïdien – Antimicrobien – Bovins – Diclofénac – Oxytétracycline – Pharmacocinétique – Résidu.



Estudio farmacocinético y evaluación de los residuos tisulares tras la administración de una combinación a base de oxitetraciclina y diclofenac en bovinos

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Resumen

Ocho bovinos machos recibieron una dosis única de una combinación oxitetraciclina/diclofenac a razón de 20 mg/kg de oxitetraciclina y 0.5 mg/kg de diclofenac por la vía intramuscular. Se obtuvieron muestras de sangre a diferentes tiempos hasta las 168 h post-administración. Posteriormente los animales experimentales fueron sacrificados de a pares semanalmente y hasta los 28 días post-administración. Se obtuvieron muestras de músculo, zona de inyección, hígado, riñón y grasa.

Las concentraciones de oxitetraciclina y de diclofenac en las muestras obtenidas se determinaron por cromatografía líquida de alta resolución. El análisis cinético se realizó por regresión lineal utilizando el programa CSTRIP. Oxitetraciclina alcanzó una concentración plasmática máxima (C_{max}) de $3.89 \pm 1.48 \mu\text{g/ml}$ con una prolongada semivida de eliminación ($T_{1/2\beta}$: $47.73 \pm 18.33 \text{ h}$). El perfil plasmático de diclofenac se caracterizó también por una alta C_{max} ($577.62 \pm 238.40 \text{ ng/ml}$), y una $T_{1/2\beta}$ prolongada ($30.48 \pm 9.42 \text{ h}$). Si bien las concentraciones de oxitetraciclina se midieron hasta los 21 días post-administración en hígado y tejido graso, diclofenac no pudo cuantificarse en ninguno de los tejidos ensayados a los 21 días. La prolongada semivida de eliminación presentada por el diclofenac, fue un hallazgo inesperado, ya que su $T_{1/2\beta}$ en el hombre es de solo 1.1 h.

Palabras clave

Antiinflamatorio no esteroide – Antimicrobiano – Bovino – Diclofenac – Farmacocinética – Oxitetraciclina – Residuo.



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