TISSUE DEPLETION RESIDUES IN FATTENING RABBITS FED DURING 28 DAYS WITH FEED SUPPLEMENTED WITH 40 PPM OF TIAMULIN HYDROGEN FUMARATE (TIAMUTIN® PREMIX 100)

Pérez de Rozas A.M.[1], Vives N.[2], Sitjar M.[3], García J.[3], González J.[1], Aloy N.[1], Badiola I.[1]*

[1]CReSA (UAB-IRTA), Campus de Bellaterra, Edifici CReSA, 08193-Bellaterra, Barcelona, Spain
[2]RESERCO, Reus, Tarragona, Spain
[3]NOVARTIS Sanidad Animal SL, Barcelona, Spain
*Corresponding author: ignacio.badiola@cresa.uab.es

ABSTRACT

Thirty rabbits were medicated with feed supplemented with 39 ppm of tiamulin, equivalent to 3.21±0.50 mg of tiamulin hydrogen fumarate per kg body weight and day, during 28 days, and tiamulin-derived residues (measured as 8-α-hydroxymutilin) in the muscle and liver of rabbits were evaluated at 0, 3, 6, 12 and 24 hours after the end of the medication period. A withdrawal period of 0 days can be considered, because all the residue amounts obtained in muscle and liver were below the limit of quantification in rabbits euthanized just after the end of the medication period.

Key words: Tiamulin, Withdrawal period, 8-α-hydroxymutilin.

INTRODUCTION

The purpose of this study was to provide data on the depletion of tiamulin-derived residues (measured as 8-α-hydroxymutilin) in the muscle and liver of rabbits following medication of TIAMUTIN® PREMIX 100 (Reg. N. 10.223) over 28 consecutive days at a target dose of 40 ppm, equivalent to 3 mg tiamulin hydrogen fumarate per kg bodyweight and day. The principal objective of the study was to define the time frame when the residues of 8-α-hydroxymutilin are below the established Minimum Residue Levels (MRLs) of 100 and 500 µg/kg in muscle and liver respectively (EMEA/MRL/724/00-FINAL). The study was composed of: a biological phase with oral medication of the animals with medicated feed during 28 days, and the collection of tissues and medicated feed at different time points; an analytical phase for the analysis of the tissues; a statistical phase in which the withdrawal period is calculated based on the analytical data.

MATERIALS AND METHODS

Animals and origin

Thirty six New Zealand x Californian rabbits were used in this study. The animals were delivered on 22nd December 2006 from Granja Cunícola MDL San Bernardo at Tulebras (Navarra, Spain). Only healthy animals, with the same number of males and females, were included in the study. The animals arrived to the experimental farm one week before the beginning of trial to allow for the adaptation of rabbits to the new farm conditions. All the animals had more than 0.75 kg body weight. The animals were identified by the number of the cage used for their accommodation. Each box was labelled by a number from 1 to 36. The weight of the animals was determined at the beginning of the medication period, and recorded in the raw data file of the study. The animals were individually weighed once a week on day 0, 7, 14 and 28 (end of the medication period). Physical examination during the first days of the acclimatization period and during all the medication period was performed. One animal (cage 32) died the second day of the trial with clinical signs of colibacillosis. No clinical signs or adverse effects were observed in the other rabbits.

Housing and climate conditions

The rabbits were housed individually in cages (40x60x40 cm) in a fattening farm of IRTA Experimental Unit at El Prat de LLobregat (Barcelona). Each cage was equipped with a feeder and a drinker. The room had natural ventilation and daylight illumination.

Feeding

Control feed:
Identification: Green label
Origin: Nutritional Animal Department of IRTA (CONSTANTÍ)
Composition: Standard complete pelleted rabbit feed, without antimicrobials and anti-coccidials.
Medicated feed:
Identification: Red label
Origin: Nutritional Animal Department of IRTA (CONSTANTÍ)
Composition: Standard Complete pelleted rabbit feed supplemented with TIAMUTIN PREMIX 100 at target dose of 40 mg of tiamulin hydrogen fumarate/kg of feed (40 ppm), and without anti-coccidials. The pelletisation temperature was adjusted to a mean temperature of 65ºC and a maximum temperature of up to 80°C. Thirty kg paper bags, labelled with colour, were filled with the pelleted feed. The feeds used were analysed to confirm the tiamulin concentration. Feed was supplied *ad libitum*.

Experimental groups

Group	Animals	Dose	Administration period
Medicated group	30	40 ppm	28 days
Control group	6	--	28 days

Feed consumption and dose control

At the beginning of the mediation period each feeder was filled to obtain a weight of 4.00 kg (feeder+feed) and this data were annotated in the corresponding field sheet. In the first hour of the morning the weight of each individual feeder was measured and was annotated in the corresponding field sheet. The difference between the weight of the previous day and the actual weight was the amount of feed consumed by the animals. The feeders were refilled to reach 4 kg when necessary. After 28 days of medication, the medicated feed was replaced with control feed. This moment was the 0 hours of the withdrawal period, and was the first time for organ collection.

Allocation to slaughter groups

From the moment of the withdrawal of the medicated feed until the end of the experimental period, 6 animals of the medicated group (3 males and 3 females), with the exception of the 24 h group that included 5 animals (2 males and 3 females), were sacrificed at different times, to collect tissue samples for analysis of the marker residue levels. The unmedicated animals (3 males and 3 females) were euthanized the day before the end of the medication period.

Time of sacrifice	-24 hours	0 hours	3 hours	6 hours	12 hours	24 hours
Animals of medicated group	0	6	6	6	6	5
Animals of control group	6	-	-	-	-	-

Because feed consumption, and therefore tiamulin intake, was continuously monitored, the five animals with the lowest feed consumption were included in the 24 hours group (animals 14, 20, 29, 30, and 31). The remainder 24 animals, with a tiamulin intake higher than the expected amount of 3 mg/kg bw/day, were randomly distributed between the other withdrawal period groups: rabbits 4, 6, 11, 19, 25, and 33 to 0 hour group, animals 7, 8, 12, 16, 24, and 28 to 3 hours group, rabbits 5, 10, 15, 22, 26, and 27 to 6 hours group , and rabbits 9, 13, 17, 18, 21, and 23 to 12 hours group.

Euthanasia

Rabbits were weighed just before sacrifice and transferred to the slaughter facility. The animals were sacrificed by cervical dislocation to avoid any possible interference between the euthanasia products and the metabolite to analyze. The investigator and the veterinarian responsible for the study proceed, immediately after the sacrifice, to the necropsy of the animals to collect the samples that were used in the analysis of residues. The following tissues were collected from each rabbit in the following order: Muscles (leg, back), total approx 100 g; liver (minus gall bladder) whole organ.

Preparation of tissue specimens

The collected tissues were divided in three aliquots and were deposited in three different bags of sampling. The bags were labelled with the number of trial, the animal number, the collection time, and type of tissue. All plastic bags containing tissue specimens were deep frozen (-19±2°C) immediately and kept frozen until shipment of tissues to the analytical laboratories in Australia (2 sets). Retention specimens (one set) were kept frozen at the test facility until the study completion date, and destroyed with the agreement of the Sponsor. In a previous study, the stability of metabolites in frozen tissues was confirmed for a period of up to 10 weeks.

Analysis of specimens

Liver and muscle specimens were analysed for the tiamulin marker residue (the sum of metabolites which may be hydrolysed to 8-α-hydroxymutilin) by Analytical Method NVR/079-01R. Detection and quantification were conducted by liquid chromatography-tandem mass spectrometry. The limits of quantification (LOQ) are 100 and 50 µg/kg for liver and muscle respectively. In each of the series of analyses, control(s) and fortified sample(s) were included to determine losses incurred during extraction and clean-up. The source of controls was from this study or purchased from local butchers. The performance of the procedure was checked by conducting at least 5 fortifications at the LOQ and 5 fortifications at the MRL. Specimens from all control study animals were analysed to estimate the limit of detection (LOD).

Statistical analysis

All depletion analyses were carried out with analytical data corrected for recovery prior to any calculation. Because all the residue data were below the quantification levels, no statistical method could be applied for the analysis of the depletion residues. For other parameters, summary statistics including arithmetic mean, minimum, maximum, standard deviation and median were calculated.

RESULTS

Feed consumption/animal body weight ratio

The average rates between daily feed intake and daily body weight for the unmedicated and the medicated rabbits are shown in Figure 1. The average rate between daily feed intake and daily body weight in the medicated rabbits was 8.22±1.29% kg of feed*100/kg bw/day. The average rate between daily feed intake and daily body weight in the unmedicated rabbits was 8.08%±1.35% kg of feed*100/kg bw/day.

Tiamulin intake

The average intake of tiamulin in the medicated rabbits was 3.21±0.50 mg/kg bw/day, an intake in accordance with the expected value. The average intake of tiamulin of rabbits of H0 group (animals 4, 6, 11, 19, 25, and 33) was 3.25±0.43 mg/kg bw/day. The average intake of tiamulin of rabbits of H3 group (animals 7, 8, 12, 16, 24, and 28) was 3.29±0.49 mg/kg bw/day. The average intake of tiamulin

of rabbits of H6 group (animals 5, 10, 15, 22, 26, and 27) was 3.26±0.46 mg/kg bw/day. The average intake of tiamulin of rabbits of H12 group (animals 9, 13, 17, 18, 21, and 23) was 3.29±0.40 mg/kg bw/day. The average intake of tiamulin of rabbits of H24 group (animals 14, 20, 29, 30, and 31) was 2.89±0.63 mg/kg bw/day.

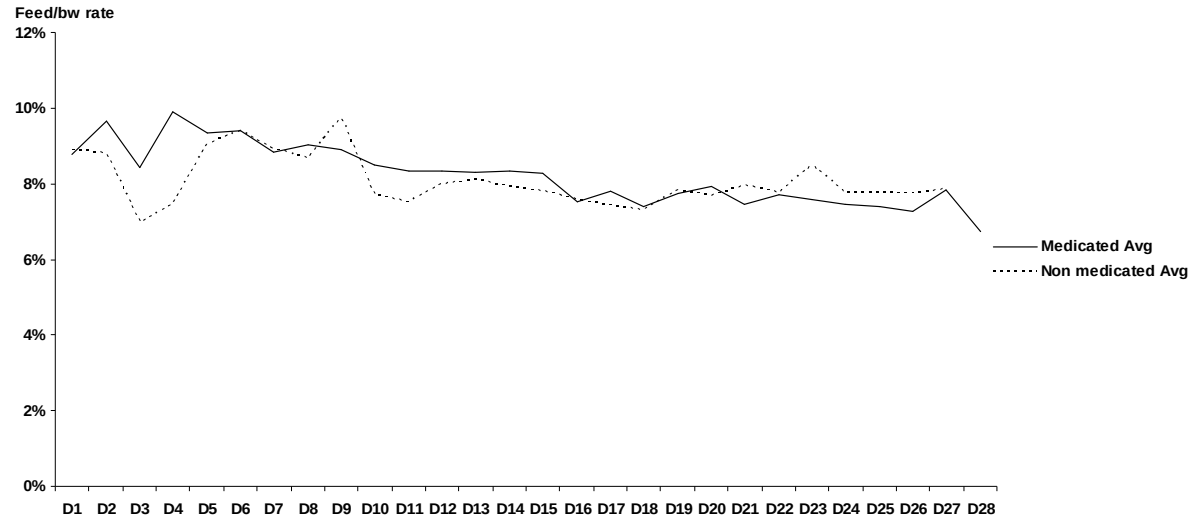

Figure 1: Average rates between daily feed intake and daily body weight for the unmedicated –discontinuous line– and the medicated rabbits –solid line– (D1-D28: day of trial)

Residue amount in rabbit tissues

The Table 1 summarizes the concentration, in µg/kg of tissue, of 8-α-hydroxymutilin determined in rabbit tissues. The residue amount of all the analysed tissue samples was below the limits of quantification of 50 mg/kg of tissue, for muscle, or 100 mg/kg of tissue, for liver.

Table 1: Residues of 8-α-hydroxymutilin determined in rabbit tissues (LOQ: limit of quantification, MRL: maximum residue level)

Time post treatment	Animal No.	Muscle Concentration (µg/kg)	Liver Concentration (µg/kg)
control	1	<50	<100
	2	<50	<100
	3	<50	<100
	34	<50	<100
	35	<50	<100
	36	<50	<100
0 hour	4	<50	<100
	6	<50	<100
	11	<50	<100
	19	<50	<100
	25	<50	<100
	33	<50	<100
3 hours	7	-*	<100
	8	-	<100
	12	-	<100
	16	-	<100
	24	-	<100
	28	-	<100
	LOQ	50	100
	MRL	100	500

*Samples were not analysed

CONCLUSIONS

The trial was conducted on 36 rabbits, with an average body weight of 1.38 kg at the beginning of the experimental period. Thirty rabbits were medicated with feed supplemented with 39 ppm of tiamulin, equivalent to 3.21±0.50 mg of tiamulin hydrogen fumarate per kg body weight and day, and six rabbits were non-medicated. No adverse reactions were observed during the experimental period, and only one medicated animal died by intestinal colibacillosis at the third day of trial.

The tiamulin intake of the medicated rabbits was 3.21±0.50 mg of tiamulin hydrogen fumarate per kg body weight and day, lightly higher, but no significantly different, than the expected value. Because the real tiamulin intake was higher than the expected amount, the trial conditions can be considered as valid for tissue residue analysis. All the residue amounts obtained in muscle and liver were below the limit of quantification in rabbits euthanized just after the end of the medication period (0 hour group) or later (3 hours group). In all cases clearly bellow the MRL fixed in EMEA/MRL/724/00-FINAL.

The overall results obtained in this trial permit to recommend a withdrawal period of 0 days, for rabbits medicated during 28 days with feed supplemented with 39 ppm of tiamulin, equivalent to 3.21±0.50 mg of tiamulin hydrogen fumarate per kg body weight and day.

REFERENCES

Atkinson A.C. 1985. Plots, Transformations and Regression. An introduction to graphical methods of diagnostic regression analysis. *Oxford statistical science series, Oxford University Press, New York, USA.*

EMEA 1996. Approach Towards Harmonization Of Withdrawal Periods, The European Agency for the Evaluation of Medical Products. *Committee for Veterinary Medical Products (EMEA/CVMP/036/95).*

Graf U., Henning H.J., Stange K., Wilrich P.T. 1987. Formeln und Tabellen der angewandten mathematischen Statistik, *3rd ed., Springer Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, 1987.*

NVR/079-01R 2004. 8-α-Hydroxymutilin: A method for determination of residues in porcine liver using LC-MS. *Huntington Life Sciences.*