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Depletion of cefquinome from rabbit tissues

Sara T. Elazab[1-3], Mohamed G. Gabr[1], Magdy S. Amer[1], Sameh M. El-nabtity[2], and Walter H. Hsu[3]

[1]Department of Pharmacology, Faculty of Veterinary Medicine, Mansoura University, El-Mansoura, Egypt

[2]Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Egypt

[3]Department of Biomedical Sciences, College of veterinary Medicine, Iowa State University, Ames, Iowa, USA

ABSTRACT: The current study was carried out in 24 New Zealand white rabbits (2.0-2.5 kg) to evaluate cefquinome residues in their tissues (kidney, liver, and muscle) following intramuscular (IM) administration of 2 mg/kg of body weight, once daily for 3 consecutive days. The solid phase extraction and high performance liquid chromatography were used to determine cefquinome concentrations in tissue samples. We found that cefquinome was highly concentrated in the kidney followed by the liver, while traces of it were detected in muscles. Cefquinome was not detected in kidney on the 24th day post-administration, while it disappeared from liver and muscle tissues on 7th and 3rdday post-administration, respectively. The withdrawal periods were established based on European UnionMaximum Residue Levels (EU MRL) using the statistical method (95% tolerance limit and 95% confidence) stated in the guidance and the withdrawal time calculation program WT1.4 which was developed by Germany and adopted by the Committee for Veterinary Medicinal Products (CVMP) of EU. The suggested preslaughter periods are: 10 days for kidney, 2 days for liver and 1 day for muscle.

KEYWORDS: Cefquinome; Residue, Withdrawal time; HPLC; EU MRL.

1 INTRODUCTION

The economic importance of rabbits has enormously increased as they are raised for many purposes. Rabbits are considered a good source of palatable meat for its higher protein, water and lower fat contents than other mammalian meats [1]. Rabbit fur is used for clothing decoration, in addition to its use as ideal laboratory animals [2].

It is very important to protect rabbits from many infectious diseases, which can be achieved by using antibiotics. These antibiotics, despite their effectiveness, can leave residues in the treated animals and contaminate their edible parts, e.g., the muscle meat. Environmental and human health risks are associated with these residues *[3]*

Cefquinome is a member of the fourth generation cephalosporins, which is used in animals only [4]. It has broad-spectrum antibacterial activity against clinically important bacteria such as *Streptococcus spp, Staphylococcus spp, Pseudomonas spp, E. coli,* and Gram positive anaerobes [5]. Cefquinomehas been extensively used for the treatment of respiratory infections caused by *Pasteurellamultocida* and *Haemophilusparasuis*in cattle and pigs [6].

The pharmacokinetics of cefquinome have been reported in rabbits ([7], [8]). In addition, tissue residues of cefquinome have been investigated in swine and chickens ([9], [10]). However, there have been no reports available on residues of cefquinome in rabbit tissues. Therefore, the aim of the present work was to evaluate cefquinome residues in tissues (liver, muscle, and kidney) in rabbits treated with this drug. Based on these data, one could establish the withdrawal time of cefquinome. This information will provide assurance that consumers will not be exposed to significant levels of cefquinome.

Corresponding Author: Walter H. Hsu

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

A total of 24 New Zealand white rabbits of both sexes were used in this study. The animals were acclimated and did not receive any drug treatment for at least 15 days before the study. They were randomly divided into 2 groups, one group of 6 rabbits served as controls (they were used for preparation of blank sample and spiked sample for method validation); the second group of 18 rabbits were injected with cefquinome at a dose regimen of 2 mg/kg once daily for 3 consecutive days ([7], [9], [10]).

2.2 MATERIALS

Cefquinome sulfate (COBACTAN®, 2.5%) was produced as suspention, each ml of the suspension contains 29.64 mg cefquinome sulfate (equivalent to 25 mg cefquinome) from Intervet International Company, Cairo, Egypt. The cefquinome reference standard was supplied by Intervet International Company, Cairo, Egypt. Acetonitrile (CAN) and methanol (HPLC grade) were purchased from J.T.Baker (Deventer, Netherlands).Water used for HPLC analysis was purified through a Milli–Q system (Waters Corp., Milford, MA).

2.3 TISSUE SAMPLES

Tissue samples (liver, muscle, and kidney) were obtained from 3 rabbits each on the 1^{st},3^{rd}, 7^{th}, 14^{th}, 21^{st} and 24^{th} day post-administration of cefquinome. Fifty grams of chest muscle, 15 g of liver and 3 g of kidney samples were obtained from each rabbit and were frozen at -20ºC until assayed.

2.4 EXTRACTION OF CEFQUINOME FROM TISSUE SAMPLE

Extraction of cefquinome from tissue samples was performed as described by [11].

2.4.1 SAMPAL PREPARATION FOR SOLIPHASE EXTRACTION

A 10-gm sample was mixed with 40 ml of pH 5, 0.05 M ammonium acetate buffer (TAC5) and 10 ml isooctane. The sample was homogenized by vortexing and rotator agitation. Homogenized samples were centrifuged at 2,400 x *g* for 10 min. Isooctane was eliminated and the supernatant was transferred into a clean 50-ml polypropylene tube.

2.4.2 SOLID PHASE EXTRACTION

A C18 SPE cartridge was attached to vacuum manifold. Each cartridge was conditioned with: 5 ml methanol, 5 ml ultra-pure water and finally 5 ml TAC5. The supernatant was poured immediately into the reservoir of the cartridge, the sample was pulled through the cartridge with vacuum. The cartridge was washed with 2 ml of TAC5, then 1 ml of ultra-pure water and finally 1 ml of the washing solution (demineralized water: acetonitrile = 90: 10; v/v). Air was drawn through the cartridge for 5 min. The reservoir was then removed from the cartridge and washes were discarded. A clean 5-ml tube was placed under the cartilage, 2 ml elution solution (80/20; v/v) was added to the cartridge and was eluted at 3 ml/min, and 400 µl acetonitrile was evaporated at 50°C under nitrogen stream. The elute volume was adjusted to 1.6 ml with TAC 7 and the pH was adjusted to 7.0 with 0.1 N NaOH.

2.5 CONDITION OF LIQUID CHROMATOGRAPHY

High Performance Liquid chromatography (HPLC) Agilent Series 1050 quaternary gradient pump, Series 1050 auto sampler, Series 1050 UV Vis detector, and HPLC 2D Chemstation software (Hewlett-Packard, Les Ulis, France) were used. The reverse phase analytical column, a C_{18} (250 mm x 4.5 mm; i.d., 5 µm), was from Agilent Co. The HPLC mobile phase was 0.02 M KH_2PO_4 : acetonitrile = 90 : 10. The injection volume was 100 µl. The flow rate was set at 1 ml/min. Column temperature was 45°C and multi-wavelength detector (MWD) was set at 267 nm.

Quantification of residues in the samples was performed and calculated from area under the curve, and extrapolated automatically by the software (HPLC 2D Chemstation software).

The withdrawal periods were determined using the withdrawal time calculation program WT 1.4 which was developed in Germany and adopted by the Committee for Veterinary Medicinal product (CVMP) of EU.

2.6 STATISTICAL ANALYSIS

Data are expressed as mean ± standard error (SE). Data were analyzed using analysis of variance. Mean comparison was performed using Tukey's test and significant difference were set at $P<0.05$. These calculations were performed using Prism 5.0 (Graph Pad, USA)

3 RESULTS AND DISCUSSION

3.1 STANDARD CURVE CONSTRUCTION

The cefquinome calibration curve was prepared at concentrations of 0.05, 0.2, 0.5, 1 and 2 µg/ml.

The calibration curve was calculated by linear regression equation as y = 0 .08342x + 0.2763 where y symbol indicates the area under peak and x symbol indicates concentrations of cefquinome. Linearity existed within range of 0.05 and 2 µg/ml with a correlation coefficient of r^2=0.998.

3.2 RESULTS OF TISSUE RESIDUES

The results of cefquinome in rabbit tissues at different times are shown in Table 1. The data showed that the mean cefquinome residue concentrations in rabbits' kidney was 0.689 µg/gm on Day 1 post-administration. The renal cefquinome concentration declined gradually until it reached 0.017 µg/gm on Day 21post-administration. Cefquinome was not detectable in the kidney on Day 24 post-treatment. The limit of detection was 0.008 µg/gm of tissue.

Table 1. Concentrations of cefquinome in kidney, liver, and muscle of healthy rabbits (µg/gm) on different days following IM administration at 2 mg/kg once daily for 3 consecutive days.

Days post administration	Residue level (µg/gm)		
	Kidney	Liver	Muscle
1	0.689 ± 0.043	0.107 ± 0.008	0.015 ± 0.001
3	0.344 ± 0.024	0.033 ± 0.002	ND
7	0.198 ± 0.003	ND	ND
14	0.039 ± 0.001	ND	ND
21	0.017 ± 0.001	ND	ND
24	ND	ND	ND

[a] *Values are shown as mean ± SE for 3 independent samples (n = 3).*
[b] *ND, not detectable (<0.008 µg/gm).*

The data showed that the concentrations of cefquinome in liver tissues of treated rabbits were 0.107 µg/gm and 0.033 µg/gm on Day 1 and Day 3 post-treatment, respectively. On the other hand, the residue of cefquinome in the liver of treated rabbits was not detectable on Day 7 post-treatment. Moreover, as shown in Table 1 and Figure 1, there were significant differences in the cefquinome concentration between kidney and liver ($P < 0.001$) on the 1st, 3rd and 7th day post-administration; no significant difference of the cefquinome concentration were found between liver and muscle on the 1st and 3rd day post-administration ($P > 0.05$). The cefquinome concentration in the muscle was 0.015 µg/gm on Day 1post-treatment. On the 3rd day after the end of treatment, cefquinome concentration was not detectable in the muscle. These findings are consistent with those of a previous study in chickens [10]. These authors found that following repeated IM administrations of 2 mg/kg cefquinome every 24 h for 3 consecutive days in chickens, the highest drug concentration was detected in the kidney and liver, while the lowest drug concentration was found in the muscle [10]. In addition, it was reported that the highest tissue concentration of cefquinome was present in the kidney and liver of chickens [12]. These findings suggested that cefquinome is excreted mainly by the kidney ([13], [14]). The MRLs permitted by the European Agency For The Evaluation Of Medicinal Products Committee For Veterinary Medicinal Products, for cefquinome are 0.05 µg/gm, 0.1 µg/gm and 0.2 µg/gm in muscle, liver and kidney, respectively of bovine and porcine, species [15]. As shown in Table 1 and figures 3 and 4, the concentrations of cefquinome resdiues in kidney and liver from rabbits IM administered 3 times at a dose of 2 mg/kg of body weight with 24-h intervals were below the acceptable EUMRL at 10 and 2 days of

withdrawal time, respectively. The withdrawal periods were determined based on EU MRL using the statistical method (95% tolerance limit and 95% confidence) mentioned in the guidance [16], which were 9.87 days for kidney, 2.02 days for liver. As the residue level of cefquinome in muscle on Day 1 post-treatment was lower than the EU MRL, thus one day withdrawal time for the muscle can be suggested. It was reported that cefquinome completely disappeared from all tissues on the 7th day after cessation of the medication in chickens [12]. Furthermore, it was reported that the withdrawal time of cefquinome from tissues of chickens was 5 days following the last dose [17], *w*hile it was found that cefquinome concentrations in all examined tissues of swine were below the accepted MRL at 72 h post-treatment recommended by the Committee for Veterinary Medicinal Products of European Medical Evaluation Agency (EMEA) *[9].* Moreover, the withdrawal periods recommended by Merck Animal Health company were 5 days for cattle meat and 3 days for pig meat, respectively.

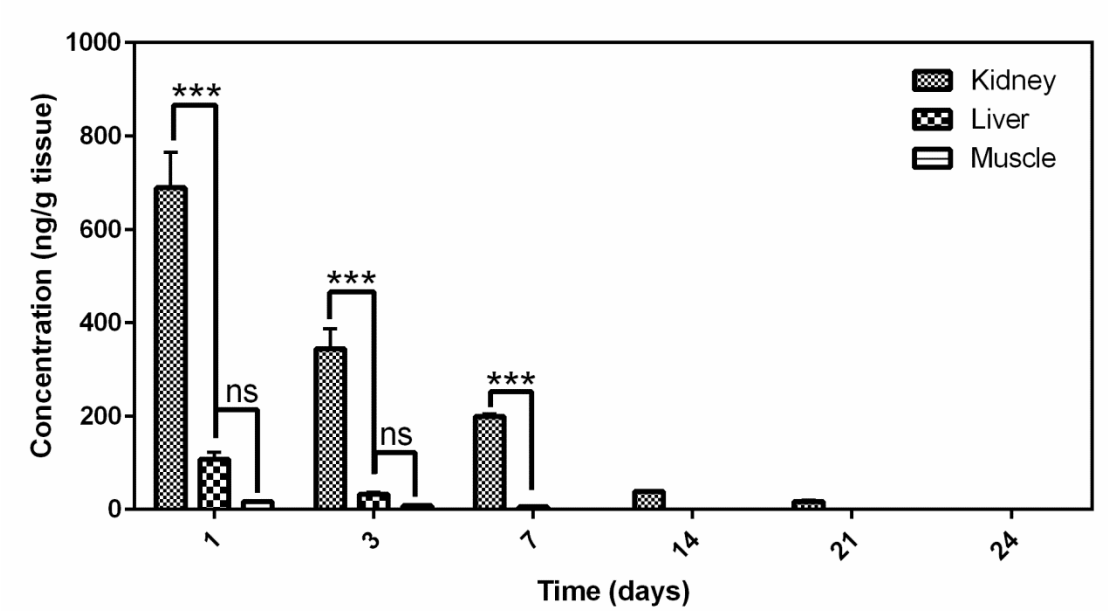

Fig. 1. Concentrations of cefquinome in the kidney, liver, and muscle of healthy rabbits (µg/gm) on different days following IM administration at 2 mg/kg once daily for 3 consecutive days. ***P<0.001; ns = no significant difference, P>0.05.

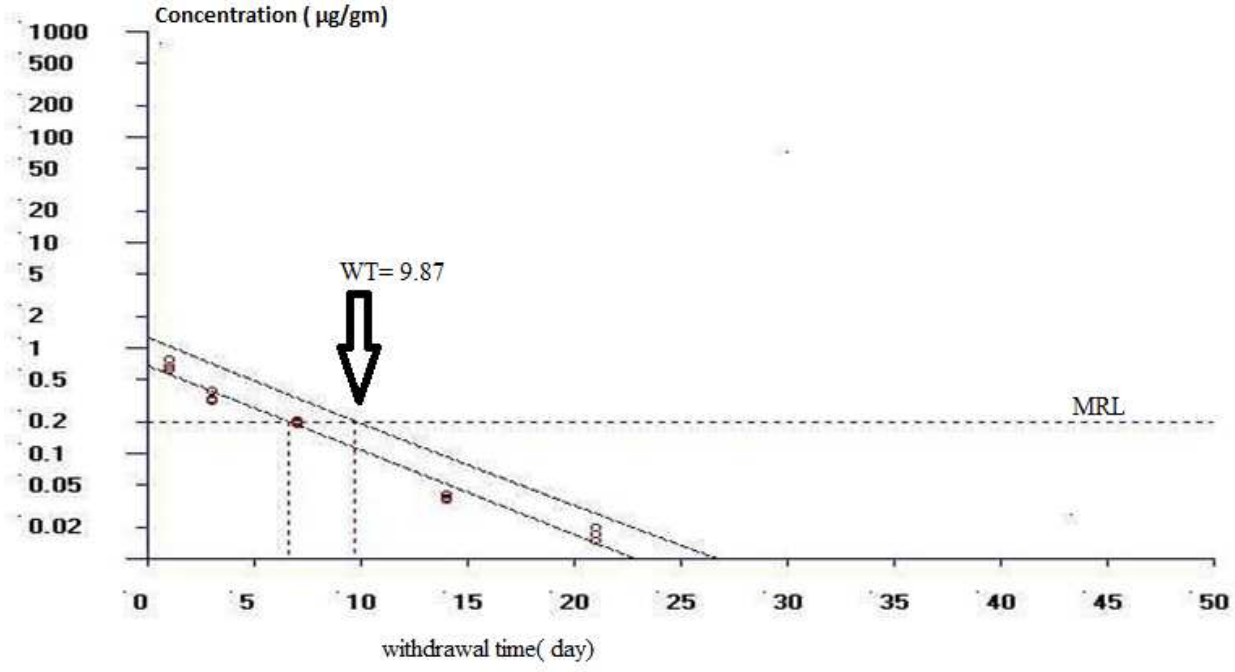

Fig. 2. Plot of withdrawal time calculation for rabbit kidney at the time when the one-sided 95% upper tolerance limit was below the EU MRL of 0.2 µg/gm.

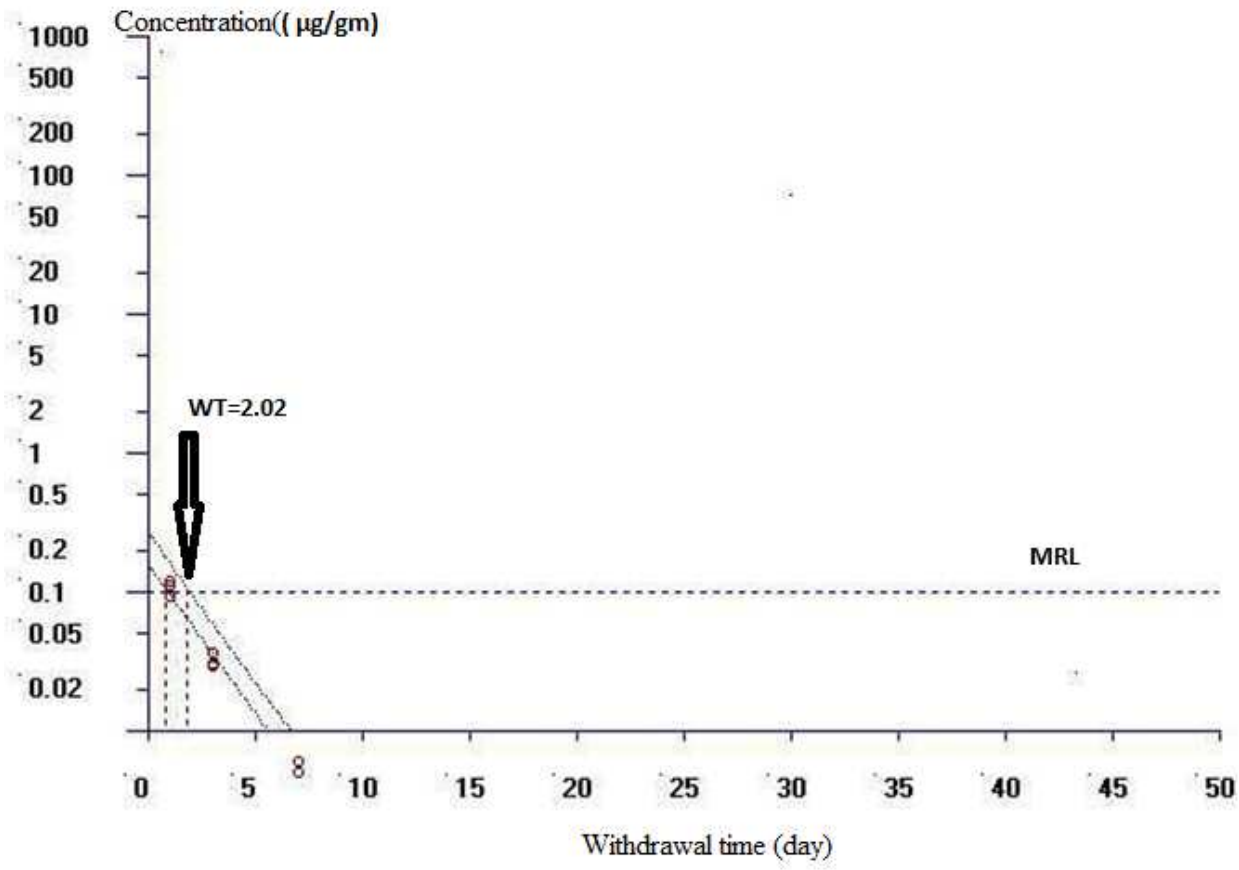

Fig. 3. Plot of withdrawal time calculation for rabbit liver at the time when the one-sided 95% upper tolerance limit was below the EU MRL of 0.1 µg/gm.

4 CONCLUSION

In conclusion, following the IM administration of cefquinome to rabbits at a dose rate of 2 mg/kg once daily for 3 consecutive days, cefquinome was highly concentrated in the kidney followed by the liver, while traces were detected in the muscle. Based on the MRLs established by regulatory agencies and statistical method suggested by EMEA, withdrawal periods of 10 days, 2 days and 1 day were recommended for the kidney, liver and muscle, respectively, which will provide assurance that consumers would not ingest significant amount of cefquinome.

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