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Detection of Tylosin Residue Levels Following Intramuscular Injection in Desert Sheep

[1]Samia A.A. Hassan, [2]Sania A.I. Shaddad, [2]I.B. ElTayeb, [3]M.A. Omer,
[4]M.H. Al-Nazawi and [4]A.M. Homeida
[1]Central Veterinary Research Laboratory, Department of Biochemistry Nutrition
and Toxicology, Alamarat, Khartoum, P.O. Box 8067
[2]Department of Pharmacology, Faculty of Pharmacy,
University of Khartoum, P.O. Box 1996, Sudan
[3]Faculty of Pharmacy, Omdurman Islamic University, Sudan
[4]College of Veterinary Medicine and Animal Resources, University of King Faisal,
P.O. Box 3498, Al-Ahsa 31982, Saudi Arabia

Abstract: A total of 12 healthy desert sheep were injected intramuscularly with multiple doses of 5 g kg^{-1} tylosin. Samples were collected at time 1, 3, 7 and 10 days after drug administrations. Tylosin was detected using microbiological method. Tylosin concentrations in different tissue were detected. High concentration of 61.78±5.83, 315.97±73.07 and 3956±645.59 μg g^{-1} were detected in liver, kidney and muscle, respectively at day 1. The highest concentrations of 19185±2966.9 μg g^{-1} were noted at the site of injection at day 1 and persisted for 10 days. Low concentrations of 1.00, 1.5±0.24 and 5.22±0.20 μg g^{-1} were noted in the liver, kidney and muscle at day 10, respectively. It is suggested that high tissue levels may create a reservoir of drug from which drug was released slowly into the circulation.

Key words: Residue, intramuscular, tylosin, sheep

INTRODUCTION

Tylosin an antibiotic of the macrolide group isolated from strain *Streptomycetes fradiae*, commonly used in food animal practice to treat pneumonia, foot rot, meterits and gram positive *Coccal* mastitis, *Mycoplasma* and anaerobic bacteria (Prescott and Baggot, 1988).

Tylosin can be administered in feed, drinking water, or injected intramuscularly for prevention and treatment of Chronic Respiratory Disease (CRD) and to improve weight gain and feed efficiency. Residues of tylosin could be found in tissue of treated animals (Delepine *et al*., 1994; De Liguoro *et al*., 1998; Prats *et al*., 2002).

Tylosin has a wide margin of safety in all species tested. Parental administration in swine has been followed by skin erythema, pruritus, rectal edema and transient diarrhea (Brander *et al*., 1982).

Side effects caused by macrolides are uncommon and only a very few of these seem to be caused by allergic mechanisms.

Several methods like microbiological assay (Nouws, 1999), Liquid Chromatography-Mass Spectrometric (LC-MS) method (Delepine, 1994) and HPLC method have been used to detect tylosin residues level in milk and tissues. However, the most suitable methods for detection of tylosin are ELISA (Draisci *et al*., 2001) and microbiological test (Althaus *et al*., 2003).

The aim of the experiment presented here was to determine the concentration levels of tylosin in certain organ after intramuscular administration in sheep.

MATERIALS AND METHODS

Animal: Twelve male's desert sheep, aged 9-12 month, weighing between 25-35 kg were used. They were housed in pens (3×3 m) and fed with balance (concentrate and forage) and water was available *ad libitum*. After two weeks adaptation period, maximum therapeutic dose of tylosin (Macrolan-200, Interchemie, Holland) was given Intramuscularly (IM) at dose of 5g kg^{-1} daily for 5 days, the animals were slaughtered and tissue samples (liver, kidney, muscle and site of injection were collected in sterile plastic bags at 1, 3, 7 and 10 days after the last administration and frozen at 0°C for drug assay by microbiological method using *Bacillus subtitilis* BFA (DSM618) (Koenon-Drieck and De Beery, 1998).

Corresponding Author: Mohammed Al-Nazawi, College of Veterinary Medicine and Animal Resources, King Faisal University, P.O. Box 3498, Al-Ahsa 31982, Saudi Arabia

Table 1: Tissue residues (μg g^{-1}) of tylosin after intramuscular administration of 5 g kg^{-1} for 5 days to twelve healthy sheep

Tissue	Day 1	Day 3	Day 7	Day 10
Liver	61.78±5.83	5.01±0.00	1.23±0.23	1.00±0.00
Kidney	315.97±73.07	32.9±1.29	3.2±0.71	1.5±0.24
Muscle	3956.9±645.59	1367.6±108.66	177.8±15.58	5.22±0.20
Site of injection	19185.0±2966.9	3959.9±645.59	4013.2±122.83	92.9±32.75

The logarithm concentration of tylosin of known concentrations were plotted versus mean iinhibition zone diameters. From calibration curve the concentration of tylosin in samples were calculated (Koenon-Dierick *et al.*, 1995; Koenon-Drieck and De Beery, 1998).

Statistical analysis: Statistical analysis of the data obtained was carried out using ANOVA with significance difference $p<0.05$.

RESULTS

Tylosin concentration in liver, kidney, muscle and site of injection following intramuscular administration of 5 g kg^{-1} for 5 successive days are recorded in Table 1.

Tylosin concentrations were observed to be different in all examined tissues. Detectable concentrations in liver were found to be significantly decreased from 61.78±5.83 to 1.00 μg g^{-1} at day 1 and day 10, respectively while the maximum concentration was observed in kidney 315.97±73.97 μg g^{-1} at day 1, then the concentrations declined, rapidly to 32.9±1.29 and 3.2±0.71 μg g^{-1}, until it was disappeared at day 10. Levels of tylosin in muscle were at 3956±645.59 μg g^{-1} at day 1 and gradually decreased to 177.8±15.58 μg g^{-1} at day 7. No residues were shown at day 10. Highest concentrations were recorded at site of injection on day 1, the concentration were persisted at high level longest at day 3 and 7 and significantly depleted to lowest concentration at day 10.

DISCUSSION

From results achieved, it is evident that tylosin residues could be detected in the liver, kidney, muscle and site of injection at day 1, 3, 7 and 10. These findings were similar to those obtained by FAO (1991).

As shown in Table 1 the concentration of tylosin residues in liver and kidney were nearly similar to those reported by Handy and Matsuoka (1978) in calves using *Sarcina lutea* as the test organism and agree with Moran *et al.* (1990) who observed that the kidney residues persisted through day 7 post treatment using HPLC method. These results give indication that tylosin was highly eliminated in kidney. Atef (1991) reported that tylosin concentrations were much higher in urine than in serum. So, the kidney might be the target tissue for tylosin residues after injectable forms.

The residues were higher and persisted longer in muscle and site of injection up to ten days. Similar observation have been described elsewhere (Moran *et al.*, 1990; Prats *et al.*, 2002) where the site of injection still contained quantifiable level of tylosin residues in calves after 21 days using HPLC method. The same observations were obtained by Nogawa (1982) who found that the residues were detectable in injection site and un-injected muscle for 25 and 3 days, respectively. Our observation were similar to that of Prats *et al.* (2002) who reported that tylosin residues in all tissues were below the quantification limits of the European Union (MRL; 50 μg kg^{-1}) at 10 and 14 days post treatment using HPLC. This could be due to the facts that the macrolide antibiotics are weak organic bases and are distributed throughout most of the body. The extensive intracellular ionization of these molecules results in ion trapping and accumulation in the intracellular fluids. Thus resulting in high tissue concentrations. These high tissue levels create a reservoir of drugs that could be slowly released into the systemic circulation and allow for a long duration of action from multiple administration of drug.

The results of these studies were different from that obtained by Nouws *et al.* (1999) who reported that tylosin residues were undetectable in meat 24 h after intramuscular administration using microbiological method and Moats *et al.* (1985) who found that residues were not detected in any tissue at 48 h after treatment using liquid chromatographic and bioassay procedures.

The difference could be due to the differences in doses, interval time of sample collection and method of detection.

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