Preparation and evaluation of veterinary 20% injectable solution of tylosin

Fouad K. Mohammad[1*], Muzahim Y. Al-Attar[1], Faris T. Abachi[2] , Ashraf S. Alias[1], Thiab A. Al-Sawah[3] and Talib A. Al-Talib[4]

1. College of Veterinary Medicine, 2. College of Pharmacy, University of Mosul,
3. The State Company for Drugs and Medical Appliances-Samarra,
4. The State Company for Drugs and Medical Appliances-Nineveh, Mosul, Iraq
*Corresponding author E-mail: fouadmohammad@yahoo.com

Abstract

A veterinary injectable aqueous solution of the antibiotic tylosin at a concentration of 20% was prepared under aseptic conditions in dark glass bottles each containing 100 ml. The preparation was intended for animal use only. It contained 200 g tylosin tartrate, 500 ml propylene glycol, benzyl alcohol 40 ml as a preservative and water for injection up to 1000 ml. The preparation was clear yellow viscous aqueous solution free from undesired particles. The preparation complied with the requirements for injectable solutions. It was active in vitro against E. coli (JM83). The preparation of 20% tylosin solution was safe under field conditions in treating sheep and cattle suffering from pneumonia at the dose rate of 1 ml/20 kg body weight, intramuscularly/ day for 3 successive days. In conclusion, we presented the know-how of a veterinary formulation of injectable solution of 20% tylosin for clinical use in ruminants.

Key words: Tylosin, Veterinary preparation, Pneumonia, Injectable solution.

Introduction

Tylosin is macrolide antibiotic produced from *Streptomyces fradiae* and related structurally to erythromycin (PlumB, 2002; Giguere, 2007). It occurs as a white to buff-colored powder with a pKa of 7.1 (Plumb, 2002; Bishop, 2005). Tylosin is slightly soluble in water, but soluble in alcohol; tylosin tartrate, however, is soluble in water (Plumb, 2002; Bishop, 2005). Tylosin, similar to erythromycin, acts as bacteriostatic agents by binding to 50S ribosome and inhibits protein synthesis of the bacteria (Bishop, 2005; Giguere, 2007). It is used in ruminants to treat various systemic infections such as bronchopneumonia, mastitis, footrot and metritis caused by susceptible micro-organisms (Aiello, 1998; Entriken, 2001; Plumb, 2002; Giguere, 2007).

Tylosin is well distributed in the body after intramuscular administration, with the exception of penetration into the cerebrospinal fluid, and it is eliminated in the urine and bile apparently as unchanged drug (Aiello, 1998; Plumb, 2002; Giguere, 2007). The elimination half life of tylosin is reportedly between 1-2 hours in ruminants (Taha et al., 1999; Giguere, 2007).The recommended therapeutic dose of tylosin in ruminants is between 10-20 mg/kg/day for 5 days, intramuscularly for treatment of various systemic bacterial infections (Aiello, 1998; Plumb, 2002; Aanonymous, 2003; Bishop, 2005).

Many commercial preparations of the injectable forms of tylosin are available in the Middle East for treating sheep, goats and cattle (Entriken, 2001; Anonymous, 2003; Bishop, 2005). The veterinary injectable dosage forms of tylosin in the current market place of Iraq and neighboring countries are mostly imported and quantities of formulation ingredients are not fully known. The present report introduces the know-how and a successful approach to prepare an injectable multiple dosage form of tylosin suitable for use in ruminants.

Materials and methods

The chemicals (BP) used were tylosin tartrate, propylene glycol, benzyl alcohol and water for injection. The ingredients and their amounts of the injectable multiple dosage form of 20% tylosin are shown in table 1.

Table 1: The ingredients and their amounts of the veterinary injectable solution of 20% tylosin

Ingredients	Amount/1000 ml
Tylosin tartrate	200 g
Propylene glycol	500 ml
Benzyl alcohol	40 ml
Water for injection to make	1000 ml

Tylosin tartrate was dissolved in 500 ml propylene glycol with continuous stirring. Then, benzyl alcohol was added to the solution with continuous stirring until a clear solution was obtained, and thereafter the volume was completed with continuous stirring to 1000 ml with water for injection. The final solution of tylosin was sterilized by filtration (pore size 0.2 µm) (Blodingers, 1983; Ansel et al., 1999), and distributed into 100 ml amber-colored glass bottles. We capped the bottles and sealed them tightly.

The injectable 20% solution of tylosin was subjected to various examinations that included, sterility test on blood agar and brain-heart agar for bacteria and sabouraud agar for fungi (Ansel et al., 1999; Brooks et al., 2001), visual inspection to determine the color and any macro contaminant and concentration of the active ingredient tylosin (this assay and additional ones were done by Syphco Co. for Drugs and Chemicals, Damascus, Syria). The preparation was tested in vitro for efficacy against *Escherichia coli,* strain JM83, which is a reference strain (G.M. Weinstock, Department of Biochemistry and Molecular Biology, University of Texas, USA). Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) (Brooks et al., 2001) were calculated for *E. coli* and compared with another commercial preparation of tylosin 20% (Uvedco Co., Amman, Jordan) as a positive control.

For safety evaluation and clinical application, the preparation of 20% tylosin was injected at 1 ml/20 kg body weight, i.m./day for 3 consecutive days in sheep at the Animal House facility of the College of Veterinary Medicine, University of Mosul, and then in 50 sheep and 20 calves suffering from various types of clinical manifestations of pneumonia. The animals were monitored for any physical unexpected side effects or lethality.

The present study has been approved by the Scientific Committee of the College of Veterinary Medicine at the University of Mosul (Iraq). All experiments complied with regulations addressing animal use, and proper attention and care have been given to the animals used in the study.

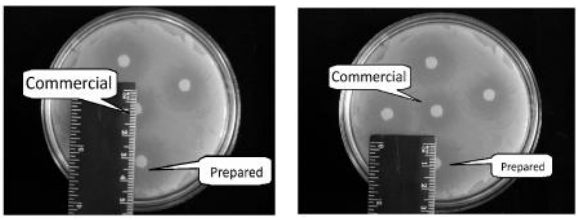

Figure-1 : Zone of bacterial *(E.coli)* inhibition by the prepared and Commercial (control) 20% tylosin solutions.

Results

The content of atropine sulfate complied with the requirements of this preparation with a range of 97-110%. The prepared dosage form of 20% tylosin was clear yellow viscous aqueous colorless solution; it was sterile, free from undesired particles. It was active in vitro against *E. coli*. The prepared dosage form of 20% tylosin was comparable with the commercial preparation used when tested for in vitro antibacterial activity. The MIC was 12.5 µg/ml for both the prepared 20% tylosin and positive control (commercial preparation), whereas the MBC was 25 µg/ml for both the prepared 20% tylosin and positive control (commercial preparation). However, the zone of bacterial inhibition was 18-20 mm for the prepared 20% tylosin and 15-17 mm for the positive control (commercial preparation) (Figure 1).

The prepared dosage form of 20% tylosin appeared to be safe and clinically effective in treating sheep and calves suffering from pneumonia and none of the treated animals showed undesirable side effects or death.

Discussion

The prepared injectable dosage form of 20% tylosin was in compliance with the requirements of parenteral solutions intended for injection (Blodingers, 1983; Ansel et al., 1999; USP 2002). The specifications of the prepared solution were well within those required by B.P. (Vet.) (2000) and USP (2002). Tylosin tartrate is soluble in water and propylene glycol (Plumb, 2002; Bishop, 2005). Benzyl alcohol is used in the preparation as a preservative because of its multiple dosage form (Blodingers, 1983; Ansel et al., 1999). The prepared dosage form of 20% tylosin was similar to the commercial preparation used when tested for in vitro antibacterial activity against *E coli*. Further, the preparation was safe in sheep and calvesle when used under field conditions and the drug effectively treated the animals that suffered from pneumonia without any noticeable side effects.

Tylosin injection solutions which are used in veterinary practice in Iraq are usually imported. The present findings would be a potential contribution for the manufacture of 20% tylosin solution locally on a commercial basis, as we introduce the know-how of the product intended for veterinary use only. The prepared 20% tylosin solution should be protected from light and stored in multiple-dose air tight containers, preferably amber-colored glass, at a temperature of less than 40 °C (preferably between 15 to 30°C). Storing at a freezing point should be avoided. The shelf-life is 3 years from the date of manufacturing if kept under the above conditions (as determined by Syphco Co., Syria).

The label of the present preparation of 20% tylosin should state the it is a veterinary preparation that can be given by deep i.m. injection. at the dose rate of 1 ml/20 kg body weight daily for 3 to 5 consecutive days. It should be protect from light and kept between 15-30 oC. The expiry date of the product is usually 3 years from the date of manufacture.

Acknowledgement

This study was supported by the Ministry of Higher Education and Scientific Research, Iraq (Research grant No. 202/5).

References

1. Aiello, S. E. , ed. (1998): The Merck Veterinary Manual. Merck & Co, Inc., Whitehouse Station, NJ. pp.180-182.
2. Anonymous (2003): USP veterinary pharmaceutical information monographs – antibiotics. *J. Vet. Pharmacol. Therap.* 23, Suppl. 2, 1-271.
3. Ansel, H. C. ,et.al.(1999): Pharmaceutical Dosage Forms and Drug Delivery Systems. 7th ed. Lippincott Williams and Wilkins, Philadelphia.
4. Bishop, Y. (2005): The Veterinary Formulary. 6th ed. The Pharmaceutical Press, London. pp. 155-157.
5. Blodingers, J. (1983): Formulation of Veterinary Dosage Form. Marcel Dekker, Inc., New York.
6. BP. Vet (2000): British Pharmacopoeia (Vet.). The Stationary Office, London, UK.
7. Brooks, G. F., J. S. Butel, S. A. Morse (2001): Medical Microbiology. Lange Medical Books, New York.
8. Entriken, T. L. (2001): Veterinary Pharmaceuticals and Biologicals. 12th ed. Veterinary Medicine Publishing Co., Edwardsville, Kansas, USA. pp. 2204-2208.
9. Giguere, S. (2007): Macrolides, azalides, and ketolides. In: Antimicrobial Therapy in Veterinary Medicine, 4th ed. (Giguere, S., J. F. Prescott, J. D. Baggot, R. D. Walker, P. M. Dowling, Eds.) Blackwell Publishing, London. pp.191-205.
10. Plumb, D. C. (2002): Veterinary Drug Handbook. Iowa State Press, Ames, IA. pp. 631-633.
11. Taha, A. A., H. A. Elsheikh, A. E. Khalafalla, I. A. M. Osman, A. Salam Abdullah (1999): Disposition kinetics of tylosin administered intravenously and intramuscularly in desert sheep and Nubian goats. Vet. J. 158, 210-215.
12. U.S.P. (2002): United States pharmacopeia. 25th ed. United States Pharmacopeial Convention, Rockville, MD.

****