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ACUTE PER ORAL TOXICITY OF TILMICOSIN – SUBSTANCE "BIOVET" AD IN WHITE RATS (FISCHER – 344)

Metodi Petrichev

University of Forestry, Sofia, Bulgaria
E-mail: metodi.biochemystri@gmail.com

ABSTRACT

The acute oral toxicity of Tilmicosin – a substance "Biovet" in rats, was studied. 42 male white rats, Fischer 344 line, weighing 160–180 g, were treated with 10% aqueous solution of Tilmicosin phosphate Biovet using a gastric tube. For each dose (500, 1000, 1500, 2000, 2500, 3000 and 3500 mg/body weight), 6 were treated once. Rats, which were monitored for 7 days, taking into account the clinical picture of their intoxication and mortality.

Based on the Litchfield-Wilcoxon method used in the test, an average lethal dose of LD50 2200 (2860 ÷ 1692) mg/body weight (p = 0.05) and relatively lethal doses of LD16 = 1142 and LD84 = 3256 mg/body weight were determined, which classify the product as low toxic.

The results regarding the acute toxicity of Tilmicosin when administered orally to white rats confirm the studies conducted with the original Eli Lilly product that Tilmicosin has low toxicity when administered for oral use – LD50 > 2000 mg / body weight (Piroozi, K.S. et al. 1993).

Key words: tilmicosin, acute per oral toxicity, rats.

Introduction

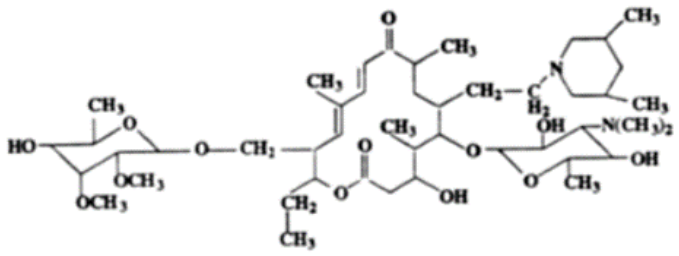
Tilmicosin is a semi-synthetic, macrolide antibiotic that has been used in the United States since 1992 (3), derived from tylosin–20–Deoxy–20 – (3,5–dimethyl piperidin – 1–41) – desmycosin (82–88% cis isomer and 12–18% trans isomer). It exhibits an antimicrobial spectrum similar to tylosin, but its effect is more pronounced against Pasteurella haemolitika and Pasteurella multocida, causes of pneumonia in cattle and sheep, and with respect to Actinobacillus pleuropneumoniae and Pasteurella multocida, causative agents of pneumonia. also against mastitis agents in sheep – Staphylococcus aureus and Mycoplazma agalactiae.

Tilmicosin is used to treat respiratory diseases in cattle, but a number of authors have argued that it can be used in horses, pigs, sheep, goats, rabbits and turkeys (Jordan F.T. et al. 1999, Mateusen B., et al. 2001, McKay S.G et al. 1996, Ramadan, A. 1997, Wakambayachi, K. and Yamada. S. 1972).

Phosphate salt (Tilmicosin phosphate) is used in the preparation of medicinal products. It is only administered orally (pigs, rabbits, chickens) or subcutaneously (cattle, calves, sheep over 15 kg), never intramuscularly and intravenously. It metabolizes slowly in the body (the usual s.c. dosage is a single dose – 10 mg / body weight).

In pigs, it is administered in the form of a premix added to the feed at a dosage of 200 to 400 ppm. The following pharmaceutical forms have been developed on the basis of Tilmicosin 'Biovet": Tilmovet 30% (solution for injection), Tilmovet 20% (premix for medicated feed), Tilmovet 20% (granules for oral administration) and Tilmovet 25% (oral solution).

The purpose of this study was to determine the LD_{50} of Tilmicosin, a „Biovet" substance, after single, oral administration in white rats.



Tilmicosin

Materials and methods

The experiment included 42 male white rats, Fischer 344 line weighing 160–180 g. All animals were reared in polycarbonate chests with dimensions 80x50x20 cm, with constant access to granular food (production of Topoli – 2004 Feed Ltd., Topoli village, providing: Crude protein – 20.80%, Calcium – 0.82%, Phosphorus – 0.63%, Energy – 2940 kcal/kg) and water. The rats were pre-treated with internal parasites with Bulmectin 0.2% – a premix containing abamectin at a dose of 0.200 mg/body weight, once with food. After a 10-day adaptation period, the rats were divided into 7 groups (6 each) and treated with Tilmicosin Biovet substance (phosphate salt), batch No. 031101. Tilmicosin was given as a 10% aqueous solution with respect to pure tilmicosin with a pH of 5.7. Groups of 6 male rats were given single oral dosing, doses with increasing gastric tube (from 500, 1000, 1500, 2000, 2500, 3000, 3500 mg/body weight). Mortality and clinical manifestations of intoxication were reported by day 7 after treatment. The mean lethal dose (LD_{50}) was determined by the Litchfield - Wilcoxon method (Litchfield Y. Y., Wilcoxon, F. 1949); the confidence intervals as well as LD_{16} and LD_{84} were determined by "probed" analysis using the same method.

All animals that died during the experiment were autopsied and examined for pathoanatomical changes. The remaining live animals, after a 7-day observation period, were euthanized and examined. The experiment was carried out in accordance with the requirements of Ordinance No. 25 of 10.06.2003 on the protection and welfare of experimental animals, promulgated, SG (BG). Issue 59 of 1 July 2003.

Results and discussion

No significant changes in the general condition and behavior of rats were observed in the 500 mg/body weight treated group. Only three of the animals showed transient, impaired locomotor activity (threshold toxicity). In the groups receiving higher doses (over 1000 mg/body weight), signs of intoxication were observed at 1 hour after treatment with rats. The clinical picture of poisoning was manifested in decreased motor activity, drowsiness, difficulty in breathing, ataxia, coma and death.

A maximum lethal dose of 3500 mg/body weight was found. The reported signs of intoxication in the experimental animals in the individual groups are presented in Table. 2. Deaths of rats receiving lethal doses (3000 and 3500 mg/body weight) occurred between the 1st and 24th hours after treatment. The intoxication survivors of the rats were fully recovered between day 5 and day 7 of Tilmicosin administration.

The processing of the results for LD_{50} is presented in Table. 1. Following the calculations, a value of LD_{50} of 2200 (2860 ÷ 1692) mg / body weight was found at p = 0.05, LD_{16} = 1142 and

LD_{84} = 3256 mg/body weight, which defined the product as low toxic. Pathologically, no visible changes in the internal organs were observed, either in the dead experimental animals or in the euthanized rats at the end of the experiment.

According to the Hodge-Sterner classification as well as the Melnikov classification, (Melnikov, N. N. 1987) Tilmicosin refers to the group of slightly toxic substances. According to the literature data, LD_{50} for Tilmicosin when administered orally to white rats (Sprague Dawley) after a fasting diet is 850 mg/body weight (Anonimus. 1999, Christodoulopoulos G. 2002, Jordan, W.H, et al. 1993, Jordan F. T., et al. 1999).

In experiments with no prior fasting diet, LD_{50} was > 2000 mg/body weight (Piroozi, K. S., 1993, Roberts, G.1999). Our results regarding the acute toxicity of Tilmicosin when given to oral rats in white rats confirm the studies conducted with the original product of Eli Lilly (USA) according to which Tilmicosin has low LD_{50} toxicity > 2000 mg/body weight when administered internally (Piroozi, K. S., et al. 1993).

Table 1: Acute toxicity studies of Tilmicosin – a 'Biovet' substance in male white rats.

Doses mg/body weight	Observed effect Num. of Experienced / Num. of dead	Observed effect		Expected effect		Difference between observed and expected effect%	Collectable on X 2
		to break through	%	to break through	%		
1	2	3	4	5	6	7	8
500	0/6	2.67	1.0	3.10	2.9	1.9	0.0130
1500	1/6	4.03	16.7	4.17	20.3	3.6	0.0310
2000	3/6	5.00	50.0	4.88	45.3	4.7	0.0100
2500	4/6	5.43	66.6	5.51	69.5	2.9	0.0045
3000	5/6	5.97	83.3	5.91	81.8	1.5	0.0015
3500	6/6	6.90	97.1	6.35	91.1	6.0	0.0046

Table 2: Reported signs of intoxication in experimental animals in the individual groups.

The dose mg/body weight	Hour/day	SIGNS OF INTOXICATION					
		Reduced motor activity	Sleepiness	Difficulty breathing	Ataxia	Coma	Died
500	Time 1–12	3/6	6/6	0/6	0/6	0/6	0/6
	Time 12–24	3/6	4/6	0/6	0/6	0/6	0/6
	Day 2	0/6	0/6	0/6	0/6	0/6	0/6
	Day 7	0/6	0/6	0/6	0/6	0/6	0/6
1500	Time 1–12	5/6	5/6	2/6	0/6	1/6	1/6
	Time 12–24	3/5	2/5	1/5	0/5	0/5	0/5
	Day 2	0/5	0/5	0/5	0/5	0/5	0/5
	Day 7	0/5	0/5	0/5	0/5	0/5	0/5
2000	Time 1–12	4/6	4/6	3/6	4/6	2/6	2/6
	Time 12–24	3/4	2/4	2/4	1/4	1/4	1/4
	Day 2	0/3	0/3	0/3	0/3	0/3	0/3
	Day 7	0/3	0/3	0/3	0/3	0/3	0/3
2500	Time 1–12	6/6	6/6	4/6	3/6	2/6	2/6
	Time 12–24	2/4	2/4	1/4	1/4	2/4	2/4
	Day 2	0/2	0/2	0/2	0/2	0/2	0/2
	Day 7	0/2	0/2	0/2	0/2	0/2	0/2
3000	Time 1–12	5/6	6/6	5/6	3/6	3/6	3/6
	Time 12–24	3/3	3/3	3/3	1/3	2/3	2/3
	Day 2	1/1	1/1	1/1	1/1	0/1	0/1
	Day 7	0/1	0/1	0/1	0/1	0/1	0/1
3500	Time 1–12	6/6	6/6	6/6	2/6	4/6	4/6
	Time 12–24	2/2	2/2	2/2	2/2	2/2	2/2
	Day 2	0/0	0/0	0/0	0/0	0/0	0/0
	Day 7	0/0	0/0	0/0	0/0	0/0	0/0

Macrolide antibiotics rarely show side effects. Some of them have been reported to cause cardiovascular effects or impaired renal function at doses above the therapeutic dose (McKay S.G. et al. 1996). High-dose tilmicosin, outside the subcutaneous route of administration in some animal species, also leads to cardiovascular toxicity (Christodoulopoulos G, et al. 2002). It causes a positive chronotropic and a negative inotropic effect, a weakening of cardiac activity and increased breathing, which was also observed in our experiments. According to some authors, the cardiovascular effect of Tilmicosin is not associated with lipid peroxidation of the heart (Modric, S. et al. 1998). Some researchers have used the creatine kinase enzyme as biochemical criteria for cardiotoxicity (Gheith, A. et al. 2015, Womble A. et al. 2006, Yazar, E. et al. 2004). Gastrointestinal malaise, vomiting, lifting, and damage to the liver may occur frequently after administration of macrolide antibiotics (Bureau Report. 1995). Tilmicosin has been shown to have a different side effect compared to other macrolidic – cardiotoxicity, depending on the animal type, route of administration and dose (Christodoulopoulos G, et al. 2002, Jordan, W. H, et al. 1993, Jordan F. T. et al. 1999, McKay S. G., et al. 1996).

Tilmicosin at a dose of 15 mg/body weight has been shown to be safe and effective in young lambs with respiratory infections (Christodoulopoulos G, et al. 2002). It has been shown that it can cause transient swelling at the injection site, acute dyspnoea, anaphylaxis, collapse and death, and these manifestations have specific relationships (Barragry, T. B. 1994, Bureau Report. 1995, Georgiev, B. et al. 2010, Jordan, W. H, et al. 1993). Some researchers claim that tilmicosin toxicity is due to positive chronotropic and negative inotropic cardiovascular effects (Christodoulopoulos G, et al. 2002, Jordan F. T. et al. 1999).

Our attempts to determine the acute toxicity of Tilmicosin "Biovet" show that the oral preparation of white rats is low toxic and does not lead to side effects. Its administration in the form of medicated feed for pigs in the prophylactically therapeutic dose (200 to 400 ppm) does not pose any toxicological risks.

Conclusion

The mean lethal dose (LD_{50}) of Tilmicosin – a Biovet substance administered once daily after 12 h fasting diet in male white rats (Fischer – 344) was 2200 (2860 ÷ 1692) mg/body weight at $p = 0.05$, $LD_{16} = 1142$ and $LD_{84} = 3256$ mg/body weight.

According to the Hodge-Sterner scale Hodge-Sterner Tilmicosin – a substance can be classified as low toxic.

The clinical picture of intoxication is characterized with decreased movement activity, drowsiness, difficulty breathing, ataxia and coma.

At the necropsy of both the dead and the euthanized surviving experimental animals have not been shown any visible changes in the internal organs.

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