Amiodarone May Prevent the Tilmicosin-caused Lethal Toxicity

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ABSTRACT

\textbf{Background}: Tilmicosin is widely used in veterinary medicine and its accidental overdose by injection may cause death via causing negative inotropy and positive chronotropy in both the treated animal and the veterinarian. In addition, there is no any antidote against to tilmicosin-caused death. Amiodarone blocks some channels in the heart, but it has much complex effect including vagotonic, bradycardic etc on the heart. Considering vagotonic and bradycardic effects of amiodarone, it has been hypothesised that amiodarone may prevent tilmicosin-caused death. The aim of this study was to determine the effect of amiodarone on the survival rate of rats in tilmicosin-caused lethal toxicity.

\textbf{Materials, Methods & Results}: Twenty female Wistar rats (body weight: 288 ± 33.8 g, age: 7-8 months) were used in this study. The study protocol was approved by the Ethical Committee. Rats received food and water ad libitum. The rats were divided into two groups containing 10 rats each. Rats in Group 1 were administered 360 mg/kg of tilmicosin in a single subcutaneous injection. Rats in Group 2 were administered 25 mg/kg of amiodarone via the tail vein at 8 min after the single subcutaneous injection of tilmicosin in a dose of 360 mg/kg. After the injections, deaths were recorded at 0, 2, 6, 10, 12 and 24 h. At the end of the 24-h period, survival/death ratio was analysed by the Chi-square test. The level of statistical significance was set at $P < 0.05$. The survival rate of Group 2 (40\%) was statistically significantly ($P < 0.025$) higher than that of Group 1 (0.0\%). In control group all rats died at 10 h after subcutaneously tilmicosin injection. In Group 2 were administered 25 mg/kg of amiodarone (intravenously) at 8 min after the single subcutaneous injection of tilmicosin in a dose of 360 mg/kg, and 2 rats died at 2 h and 4 rats died at 12 h. At the end of the experiment, all rats died in tilmicosin injected group whereas 4 rats lived in amiodarone and tilmicosin administered group. Clinically tilmicosin administered rats were observed as worse than amiodarone and tilmicosin administered group. Observed clinical signs of toxicity were fluffed feathers, ataxia, weakness in the legs, hypoactivity, lethargy.

\textbf{Discussion}: Tilmicosin, a macrolide antibiotic, is widely used in the therapy of respiratory system infections in cattle and sheep. However, tilmicosin has cause serious cardiac side effects, and after tilmicosin administration to animals or accidental self-exposure of this drug to humans, if may cause heart related side effects including chest pain, increased serum cardiac damage markers, changed electrocardiogram, and decreased antioxidant enzyme activities in the heart, serum potassium level and death, as well as . Tilmicosin causes negative inotropy and positive chronotropy. Although the mechanism of tilmicosin-induced cardiotoxicity is not fully known, the inhibitory effect of tilmicosin on the entry of calcium into the cell may cause this lethal effect. It can be speculated that the beneficial effect of amiodarone in tilmicosin toxicity may primarily depend on the potassium channel blocking, antiarrhythmic effect and other exactly not explained effects. Amiodarone may increase survival rate and may be beneficial in the treatment of tilmicosin-caused lethal toxicity. However, especially specific cellular target or other effects of amiodarone on the heart are needed to determine in the tilmicosin toxicity.

\textbf{Keywords}: tilmicosin, amiodarone, cardiotoxic.
INTRODUCTION

Tilmicosin is widely used in the treatment of respiratory system infections in cattle and sheep. However, tilmicosin may cause serious side effects associated with the heart [24]. After tilmicosin administration to animals or exposure to this drug in humans, many side effects were reported such as chest pain, changed electrocardiogram [19], increased serum cardiac damage markers [20,21,23], and decreased cardiac antioxidant enzyme activities [22], serum potassium level [1] and death [5,24]. Tilmicosin causes negative inotropy and positive chronotropy. Although the mechanism of tilmicosin-induced cardiotoxicity is not fully known, the inhibitory effect of tilmicosin on the entry of calcium into the cell may cause this lethal effect [11,12].

Amiodarone is used as an antiarrhythmic agent. It blocks sodium channels, decreases inward L-type (slow) calcium channel activity, causes asystole (1.8%) and decreases Na⁺/K⁺-ATPase activity [2,3,18]. It also has vagotonic/sympatholytic, minimal negative inotropic, bradycardic and hypotensive effects [4,13,16]. Amiodarone prolongs myocardial repolarisation associated with bradycardia without the significant potential for torsades de pointes [15]. Amiodarone has complex, although yet incompletely understood, electrophysiological properties [17]. It has been recommended in the treatment of automatic AV junctional tachycardia. In addition, when combined with macrolide antibiotics, amiodarone may prolong QTc [18]. Zhou et al. [25] have reported that amiodarone has a beneficial effect in congestive heart failure dogs. The aim of this study was to determine the effect of amiodarone on survival rate in tilmicosin-induced lethal toxicity.

MATERIALS AND METHODS

Animals and experimental design

Twenty female Wistar rats (body weight: 288 ± 33.8 g, age: 7-8 months) were used in this study. Rats received food and water ad libitum.

The rats were divided into two groups containing 10 rats each. Rats in Group 1 were administered 360 mg/kg of tilmicosin¹ (Tilmicosin-Micotil 300 inj.), in a single subcutaneous injection. Rats in Group 2 were administered 25 mg/kg [14] of amiodarone (150 mg/3mL i.v. inj.)² via the tail vein at 8 min after the single subcutaneous injection of tilmicosin in a dose of 360 mg/kg. After the injections, deaths were recorded at 0, 2, 6, 10, 12 and 24 h.

Statistical analyses

At the end of the 24-h period, survival/death ratio was analysed by the Chi-square test. The level of statistical significance was set at \( P < 0.05 \).

RESULTS

It was determined survival rates of animal and observed clinical signs of toxicity in tilmicosin (Group 1) and tilmicosin+amiodarone (Group 2) groups.

Survival rate

The survival rates of the two groups are shown in Figure 1. The survival rate of Group 2 (40%) was statistically significantly \( (P < 0.025) \) higher than that of Group 1 (0.0%). In control group all rats died at 10 h after subcutaneously tilmicosin injection. In Group 2 were administered 25 mg/kg of amiodarone (intravenously) at 8 min after the single subcutaneous injection of tilmicosin in a dose of 360 mg/kg, and 2 rats died at 2 h and 4 rats died at 12 h. At the end of the experiment, all rats died in tilmicosin injected group whereas 4 rats lived in amiodarone and tilmicosin administered group.

Clinical signs

Clinically tilmicosin administered rats were observed as worse than amiodarone and tilmicosin administered group. Observed clinical signs of toxicity were fluffed feathers, ataxia, weakness in the legs, hypoactivity, lethargy.
DISCUSSION

Macrolide antibiotics including tilmicosin and erythromycin are commonly used in veterinary medicine for the therapy of respiratory tract infections caused by Pasteurella hemolytica, P. multocida and Ar. canobacterium pyogenes etc. However, tilmicosin has more potent cardiac side effects than other macrolides (erythromycin, tylosin, tulathromycin, etc.) used in veterinary medicine [6-9,24]. In the current study, a 360 mg/kg subcutaneously administered dose of tilmicosin caused death in all rats (100%) of Group 1 (Figure 1). It has been reported that the LD$_{50}$ of tilmicosin is 185 mg/kg in male Fisher rats and 440 mg/kg (subcutaneous) in female Fisher rats. Also, the oral LD$_{50}$ of tilmicosin is 800-850 mg/kg and >2000 mg/kg in Sprague-Dawley and Fisher rats, respectively [10]. The subcutaneous dose of 360 mg/kg of tilmicosin may be accepted as the LD$_{100}$ in female Wistar rats.

The negative inotropic and positive chronotropic cardiotoxic effect of tilmicosin is well known. Although the mechanism of tilmicosin-induced cardiotoxicity is not completely understood, this lethal side effect may be due to the blockage of calcium channels in the heart [11,12]. Studies on the effect of tilmicosin on survival rates are very rare. Main et al. [12] found that dobutamine partially reversed the negative inotropic effects of tilmicosin, while propranolol, a β-adrenergic antagonist, exacerbated this effect of tilmicosin in dogs. However, to the best of our knowledge, no antidotal drug has been recommended for the treatment of tilmicosin-induced cardiotoxicity. In this study, amiodarone administration increased ($P < 0.025$) the survival rate in tilmicosin-induced lethal toxicity (Figure 1). This beneficial effect of amiodarone cannot be explained with full certainty, as the pharmacological mechanisms of amiodarone are very complex and still poorly understood [17]. It can be speculated that the beneficial effect of amiodarone in tilmicosin toxicity may primarily depend on the potassium channel blocking, antiarrhythmic effect and other exactly not explained effects.

In conclusion, the intravenous administration of amiodarone may increase survival rate in tilmicosin toxicity. However, further investigations or clinical studies are needed to determine the specific cellular target or other effects of amiodarone.

SOURCES AND MANUFACTURERS

1 Tilmicosin-Micotil 300 inj., Lilly Elanco, Istanbul, Turkey.
2 Amiodarone-Cordarone, Sanofi Aventis, Istanbul, Turkey.

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Ethical approval. The procedure was approved (number 2012/005) by the Ethical Committee of S. U. Veterinary Faculty, Konya, Turkey.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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