

ECG alterations and changes in biochemical parameters associated with experimental salinomycin toxicosis in sheep

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Summary

Salinomycin is a monocarboxylic polyether ionophore with antimicrobial properties. It is a dietary additive used as a growth promoter for ruminants and as a coccidiostat in chickens. The mechanism of action of ionophores at the cellular level is to selectively bind certain ions creating intra and extracellular biochemical disturbances. Clinical signs of ionophore intoxication are non specific and similar in all species and include tachycardia, muscle tremor, restlessness, loss of appetite, incoordination, muscular weakness and continual panting. The present study was conducted to determine the changes in ECG parameters and possible arrhythmias and their types due to experimental salinomycin toxicosis in sheep. Acute toxicity with the ionophore (0.5 mg/kg; intravenously) was induced in 6 mixed breed female sheep. A corresponding volume of sterile saline was intravenously injected in each control sheep (n=6). Blood samples were collected before and at various time intervals after the administration of either salinomycin or saline solutions. Following centrifugation, serum biochemical parameters (ALT, AST, CK, LDH and total protein) were measured using conventional laboratory methods. In both groups, the heart sounds of sheep were carefully monitored and the electrocardiogram (ECG) was recorded. Salinomycin caused a significant (P<0.05) increase in the levels of ALT, AST, LDH and CK in the experimental animals. The mean heart rate in the control group was significantly lower than that in the experimental sheep. Numerous arrhythmias such as sinus tachycardia (11 cases), ventricular premature contraction (2 cases) and T-wave inversion (3 cases) were recorded in the experimental sheep. Acute salinomycin intoxication seems to cause numerous arrhythmias in sheep which might be due to the pathological effect of the ionophore on the myocardium.

Key words: Salinomycin, Sheep, ECG, Arrhythmia, Biochemical parameters

Introduction

Ionophore antibiotics are fermentation products of various *Streptomyces* spp. (monensin, salinomycin) and *Actinomadura* spp. (maduramycin) (Butaye *et al.*, 2003). Ionophores were first described in 1964 by Moore and Pressman. They function as mobile cation carriers and have the ability to complex with and transport organic amines (Moore and Pressman, 1964). Carboxylic ionophores such as monensin, lasalocid, salinomycin, narasin, and maduramicin are

widely used as anticoccidial drugs for poultry and as a growth promoter for ruminants. Generally, ionophores have been shown to be safe and effective in the target animals receiving the recommended dosage. However, over dosage or misuse situations can lead to a series of toxic syndromes (Wilson, 1980). Salinomycin is used to prevent coccidiosis in chickens and is also added to the feed to improve feed efficiency in beef cattle and pig (Wilson, 1980; Anderson *et al.*, 1984; Kyriakis *et al.*, 2001). Due to the fact that the currently used

ionophorous anticoccidials have a narrow margin of safety and some of them are highly toxic to several animal species, the considerable concern for their safe application is entirely justified (Vaczi *et al.*, 2006). Moreover, care must be exercised in the diagnosis of ionophore toxicosis since clinical signs and pathologic lesions are not pathognomic. Although clinical signs and pathologic lesions can be of benefit for presumptive diagnosis of ionophore poisoning, confirmatory diagnosis will require consideration of differential diagnosis and laboratory assays. Toxic dose of these drugs varies and depends on the ionophore compound and the species of animals (Wilson, 1980). Because of its favorable hemodynamic profile, salinomycin has potential as a drug for increasing cardiac output, blood pressure and left ventricular force of contraction, and for improving the myocardial blood perfusion and mechanical efficiency of the heart (Ozaki *et al.*, 1982).

In the present study, the ECG parameters and the types of arrhythmias were examined in sheep intoxicated experimentally with salinomycin. In addition, changes in biochemical parameters were also considered in the intoxicated animals.

Materials and Methods

Animals

Twelve mixed breed female sheep weighing 33.1 ± 3.4 kg were stable-fed *ad libitum* with a mixture of alfalfa hay, corn silage and barley grains. For the antiparasitic control all animals were treated with albendazole (7.5 mg/kg, orally). Each animal was submitted to clinical examination and routine hematological investigation. Sheep were randomly divided into two equal experimental groups. This work was approved by the Research Committee of Shiraz University according to the animal welfare regulations.

Blood sampling and processing

Before administration of salinomycin control blood samples were collected from the jugular vein into plain vacutainers. After overnight fasting, allowing water *ad libitum*, animals were anesthetized by

intravenous injection of sodium thiopental (16 mg/kg), followed by small maintenance doses given as needed to maintain adequate anesthesia. Acute toxicity with a single IV dose of sodium salinomycin was induced at a dosage rate of 0.5 mg/kg in group one (Rajaian *et al.*, 2009b, c). Animals in the control group were injected with a corresponding volume of sterile saline solution (about 10 ml). Blood samples were collected four times (1, 3, 7 and 14 days after dosing).

The sera were separated by centrifugation at $750 \times g$ and were kept at -12°C until analysis. Biochemical analysis was carried out to measure serum total protein using the Biuret method, ALT and AST activities by the colorimetric method of Reitman and Frankel, CK by the Sigma colorimetric (modified Hughes) method, and LDH by the Sigma colorimetric method (Cabaud and Wroblewski, 1958). All the enzyme activities were measured at 37°C and results were presented in U/l. The biochemical parameters were measured using appropriate commercial laboratory kits and a RA1000 spectrophotometer.

ECG recording

The heart sounds of sheep in both groups were carefully monitored before and after the administration of either salinomycin or saline solution in order to distinguish any arrhythmia or heart murmurs. Electrocardiograms (ECGs) were recorded to evaluate the presence and types of arrhythmias. A base-apex lead was used to record the ECG by Kenz ECG recorder model 110 (Suzuken Co., Japan). This lead was attached by placing the positive electrode on the left thorax located in the fifth intercostals space at the level of the point of the elbow or at the location where the apex beat was most readily palpated. The negative electrode was attached to the skin on the right jugular furrow two-thirds of the way from the ramus of the mandible to the thoracic inlet. The ground electrode was attached to a location remote from the heart (Reef and McGuirk, 2002). A base-apex lead ECG was recorded from each animal at a paper speed of 25 mm/s and calibration of 10 mm/mV (1 cm = 1 mV).

Statistical analysis

The data are presented as mean \pm standard error (SE). Data were analysed by mixed model repeated measured analysis of variance using SPSS/PC software. Duncan's multiple range tests were performed to detect significant differences between means. All values were expressed as mean \pm standard error of mean and a value of $P \leq 0.05$ was considered statistically significant.

Results

After the administration of intravenous salinomycin, several clinical signs were noticed including inappetence, incoordination, dehydration, increased respiration rate, tachycardia, muscular weakness, salivation, continual panting, prostration and paralysis, groan, tremors, increased body temperature, heart rate, oral and nasal discharge and bruxism. One sheep from group 1 died within 48 h after the administration of salinomycin. Results of biochemical parameters summarized in Table 1 indicate various degrees of changes in several parameters in the experimental sheep. Salinomycin administration caused a significant ($P < 0.05$) increase in the levels of ALT, AST, LDH and CK. No significant change was observed in the serum total protein concentration (Table 1).

There were no cardiac murmurs on heart auscultation in the animals. ECG records indicate that, the heart rate in the control group was significantly ($P < 0.05$) lower than that in the experimental sheep (122 ± 18 beats/min versus 210 ± 36 beats/min). In the control animals, a normal heart rhythm was noticed in all cases except for sinus

tachycardia (3 cases) and sinus arrhythmia (2 cases). In the experimental sheep, however, numerous arrhythmias such as sinus tachycardia (11 cases), ventricular premature contraction (2 cases) and T-wave inversion (3 cases) were recorded (Figs. 1 and 2). All the fast heart rates were sinus tachycardia, in which all P-waves were positive, which was expected from a base apex lead.

Discussion

The anticoccidial effect of salinomycin was discovered in 1975 (Miyazaki *et al.*, 1975) and considerable data has been published regarding its beneficial and toxic effects since then (Wilson, 1980; Neuschl *et al.*, 2001). Although it is considered safe for use in the target species receiving recommended doses, it may cause deleterious side effects if overdosed or misused in bovines (Gava *et al.*, 1997), equines (Bezerra *et al.*, 1999), swines (Armen *et al.*, 1997), rabbits (Salles *et al.*, 1994), dogs (Wilson, 1980), hens (Chalmers, 1981), and other birds (Gregory *et al.*, 1992). Salinomycin tends to bind to potassium and causes disturbance in the transport of ions in the mitochondrial membrane and reduces intracellular potassium (Zinn, 1986). Salinomycin induces a decline in the level of sodium ions in the cell too, which causes mitochondrial swelling and changes in osmotic pressure. It also causes the accumulation of intracellular calcium. An increase in cytosolic calcium causes an excessive accumulation of these ions in myofibrils, causing a subsequent ATP depletion and dysfunction of oxidative phosphorylation and cell death (Zinn, 1986).

Table 1: Values (Mean \pm SEM, n=6) for serum biochemical parameters in sheep following a single intravenous administration of salinomycin (0.5 mg/kg)

Time (days)	Parameter				
	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	CK (IU/L)	Total protein (g/l)
1	309 \pm 29.9 ^{ab}	45.3 \pm 12.7*	6512 \pm 906*	253.2 \pm 89.9*	63 \pm 2
3	465.6 \pm 40.2 ^a	38.3 \pm 17.1*	9058 \pm 1048*	111.7 \pm 36.7 ^{ab}	68 \pm 1 ^a
7	425.8 \pm 70.2*	37.3 \pm 3.2 ^{ab}	4456 \pm 520*	145.7 \pm 69.4*	66 \pm 1
14	405 \pm 12.7*	49.8 \pm 5.9 ^a	3111 \pm 367 ^{ab}	255.4 \pm 125.5 ^a	66 \pm 1
Control	209.8 \pm 5.3	20.2 \pm 2.8	2134 \pm 127	62.5 \pm 9.4	63 \pm 2 ^b

^a The highest value during 14 days after administration of salinomycin, ^b The lowest value during 14 days after administration of salinomycin. * Statistically different ($P \leq 0.05$) compared to the control

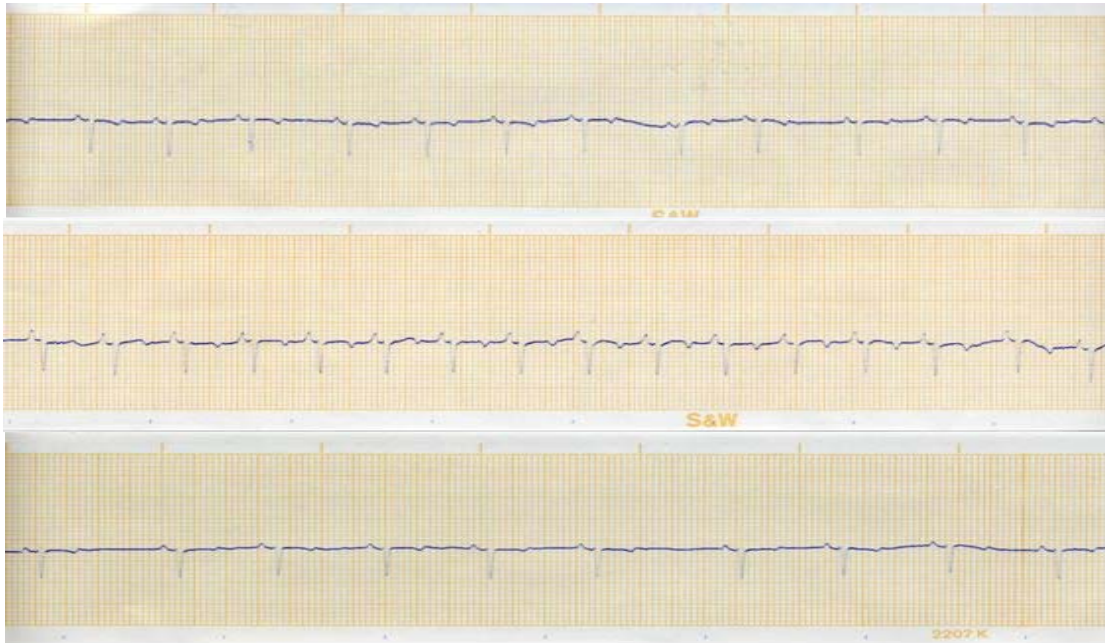


Fig. 1: ECG samples obtained prior to salinomycin administration in the control sheep illustrating sinus rhythm, sinus tachycardia, sinus arrhythmia up to down, respectively

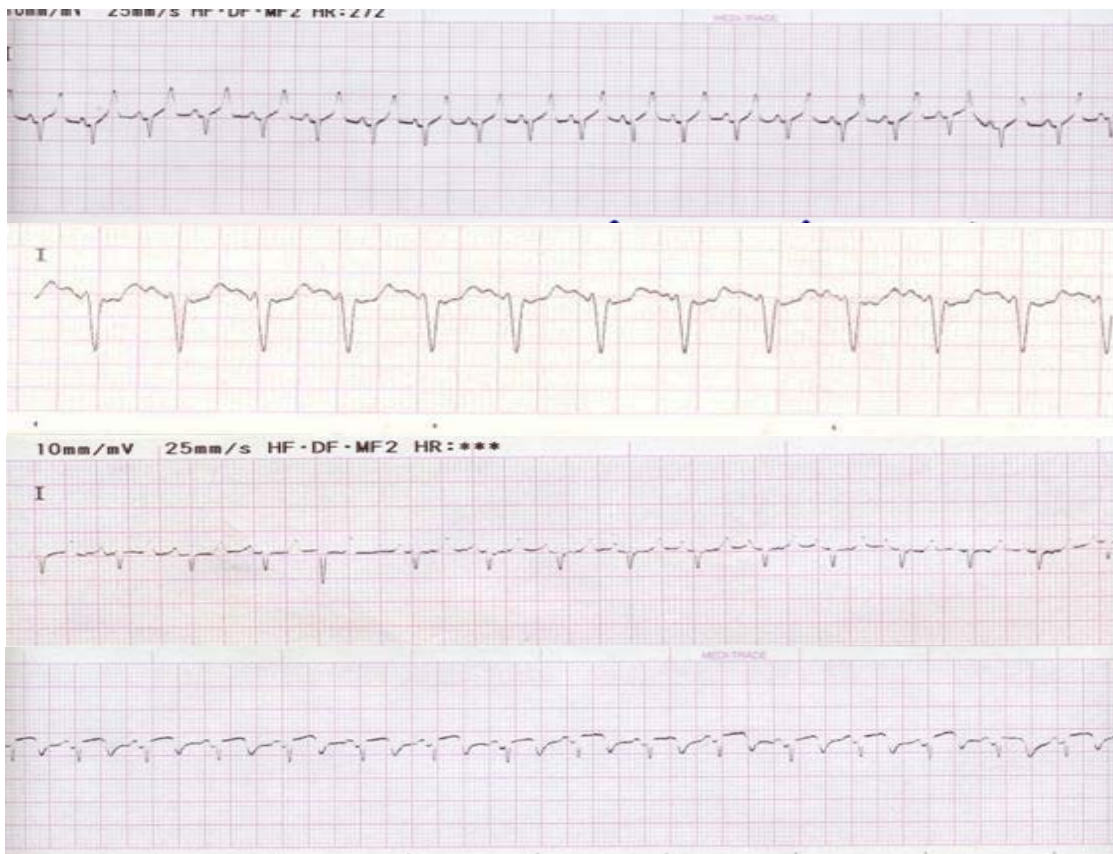


Fig. 2: ECG samples obtained after IV salinomycin administration in sheep illustrating sinus tachycardia, sinus tachycardia (another form), ventricular premature contraction, and T-wave inversion from up to down, respectively

This mechanism affects the skeletal muscles in all animals and cardiac muscles in a few.

The results of the present study illustrate the influence of salinomycin on the biochemical

parameters and ECG alterations in experimental salinomycin toxicosis in sheep. The levels of ALT, AST, CK and LDH were increased, without any significant change in the amount of serum total protein (Table 1). ALT is a cytoplasmic enzyme, and AST is both a cytoplasmic and a mitochondrial enzyme. The increase in the serum level of ALT and AST may indicate hepatic and/or myocardial damage. The increased serum activity of CK is probably due to a characteristic myopathy and probable necrosis or reversible myocyte damage (Stockham and Scott, 2002; Radostits *et al.*, 2007). A variety of insults (pathologic and iatrogenic) may damage muscle fibers and release CK from muscle fibers (Stockham and Scott, 2002). Similar biochemical findings were reported by other workers in sheep (Anderson *et al.*, 1984; Rajaian *et al.*, 2009b), goats (Dalvi and Savant, 1990), horses (Nuytten *et al.*, 1981) and calves (Rajaian *et al.*, 2009c). It has also been reported that the increase in these enzymes is possibly due to the degenerative effects of ionophores on muscles (Anderson *et al.*, 1984).

Cardiac arrhythmias are disturbances in heart rate, rhythm or conduction and can be classified based on atrial and ventricular rate, anatomic origin of the impulse, the way by which impulse is formed and conduction sequence (Reef and McGuirk, 2002). Salinomycin has been shown to exert an inotropic effect (Gupta *et al.*, 2005). This effect has been mostly attributed to the release of endogenous catecholamines (Gaide *et al.*, 1984), although Rajaian *et al.* (2009a) have proposed that the inhibition of catecholamine metabolism may also be involved.

Vasoactive effect of monensin is also reported to be due to the release of endogenous catecholamine (Ozaki *et al.*, 1982). A pronounced increase in plasma catecholamine concentration following salinomycin administration in dogs has also been reported by Fahim *et al.* (1986).

In our study, acute salinomycin intoxication seems to cause numerous arrhythmias in sheep. This result is consistent with the observation of Dzhurov *et al.* (1981) and Agaoglu *et al.* (2002) in which sinus tachycardia and supra-

ventricular tachycardia due to salinomycin intoxication was found. These findings show that the extent of myocardial degeneration is not limited to a specific site. As salinomycin form complexes with cations and mediate their transport across the cell membrane in response to diffusion gradient, mitochondrial failure and depletion of cellular adenosine triphosphate (ATP) may occur. In addition, failure of calcium ion retrieval from the cytosol and, ultimately, myofibril hypercontraction, degeneration and necrosis occur. Therefore, highly energetic tissues of the body such as myocardium and skeletal muscle are primarily affected. It should be mentioned that changes in transmembrane ion gradients and electrical potential often produce profound effects on cellular functions and metabolism (Fahim *et al.*, 1986). In the current study, various types of arrhythmias occurred in experimental salinomycin toxicosis in sheep. Some types of these arrhythmias may be related to the disturbances in impulse formation (tachycardia, sinus tachycardia and ventricular premature contraction). These results might be due to the myocardial degeneration caused by salinomycin intoxication.

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