

## Drug Interaction between Salinomycin and Exogenous Adrenaline in Dogs

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**Abstract: Problem statement:** Salinomycin is a member of ionophore compounds that may be used in ruminants and pigs as growth promoter or to prevent coccidiosis. There are some reports with respect to the *in vitro* and *in vivo* sympathomimetic cardiovascular effects of salinomycin. Available reports indicate that adrenergic impact of the ionophore is brought about by an increase in the secretion of endogenous catecholamines. In the present study, the hypothesis that salinomycin probably inhibits the biotransformation of catecholamines was examined. **Approach:** Ten female dogs weighing 22-29 kg were randomly divided into two equal groups. Two hours before general anesthesia, salinomycin with a dose of 1 mg kg<sup>-1</sup> body weight was orally administered to the dogs in group II. Adrenaline (10 µg kg<sup>-1</sup>) was intravenously infused to all animals in both groups 30 min after anesthesia. Urine was collected via urinary catheter during the experiment at different time intervals (0-30, 30-90, 90-150 and 150-210 min). Urine volume at each stage was recorded and urine samples were assayed for vanilmandelic acid (VMA) using spectrophotometric method. **Results:** Dogs in group I excreted an average of 196 µg of VMA in urine compared to 81 µg in group II during first half an h, before intravenous infusion of adrenaline. The total amount of VMA excreted in urine during 30-90, 90-150 and 150-210 min after administration of adrenaline to dogs in group I were around 426, 430 and 437 µg, respectively. The corresponding values in group II were found to be 164, 161 and 273 µg, respectively. In summary, a significant (p<0.05) decrease in the urinary excretion of VMA was noticed in dogs pretreated with salinomycin. **Conclusion/Recommendations:** It was generally concluded that the adrenergic effect of salinomycin might be elicited by a decrease in catecholamine catabolism. Measurement of catecholamine concentrations (both parent and metabolites) in serum and determination of other catecholamine metabolites in urine and serum was recommended.

**Key words:** Adrenaline, dogs, drug interaction, salinomycin

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### INTRODUCTION

Salinomycin is a monocarboxylic polyether antibiotic with antibacterial and anticoccidial properties<sup>[1-4]</sup>. Ionophores were first described in 1964 by Moore and Pressman<sup>[5]</sup>. They function as mobile cation carriers and have the ability to complex with and transport organic amines<sup>[6]</sup>. 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<sup>[7]</sup>. Monensin has also proved to

be beneficial in reversing hemorrhagic hypotension produced experimentally in dogs<sup>[8]</sup>.

Polyether antibiotics are characterized by multiple tetrahydrofuran and tetrahydropyran rings connected by aliphatic bridges. Other important features, also contributing to their mode of action include a free carboxyl function, many alkyl groups and a variety of functional oxygen groups<sup>[9,10]</sup>. The ionophore antibiotics are fermentation products of various *Streptomyces* spp. (monensin, salinomycin) and *Actinomadura* spp. (maduramycin)<sup>[11]</sup>. Salinomycin shows great preferential complexation with monovalent over divalent cations<sup>[12]</sup>. Electrophysiological effects of

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lonomycin A and monensin in cardiac Purkinje fibers have been studied by Tsuchida and Aihara<sup>[13]</sup> and Tsuchida and Otoma<sup>[14]</sup>, respectively.

Ionophores such as monensin, lasalocid and salinomycin have been shown to exert inotropic effect<sup>[8,15-20]</sup>. This effect has been mostly attributed to the release of endogenous catecholamines<sup>[21-23]</sup>. Vasoactive effect of monensin is also reported to be due to the release of endogenous catecholamine<sup>[7]</sup>. A pronounced increase in plasma catecholamine concentration following salinomycin administration in dogs is reported by Fahim *et al.*<sup>[18]</sup>. On the other hand, it has been revealed that an *in vitro* nonpolar interaction occurs between ionophore lasalocid molecule and biogenic amine such as noreadrenaline<sup>[9,17,24,25]</sup>. Therefore, a different postulation could be made to explain the cardiovascular effects of ionophores. This is based on a pharmacokinetic interaction, as ionophores may complex with endogenous catecholamines and hinder their metabolism by various enzymes such as monoamino-oxidase and catechol-O-methyl transferase. The present study was designed to test the above hypothesis. So, the effect of salinomycin on the extent of urinary excretion of one of catecholamine metabolites (VMA) was examined in dogs.

## MATERIALS AND METHODS

Ten healthy female mixed breed dogs weighing 22-29 kg were randomly divided into two equal groups. Dogs were used in accordance with the international guiding principles involving animals for scientific research at Shiraz University as well as guidelines of the Animal Welfare Act. Two hours before general anesthesia, salinomycin (22%, donated by Vetak

Pharmaceutical Co., Sirjan, Iran) was orally administered to animals in group II with an equivalent dose of 1 mg kg<sup>-1</sup> body weight. Whole egg was used as the vehicle of the ionophore. Adrenaline (10 µg kg<sup>-1</sup>) was intravenously infused to all animals in both groups 30 min after anesthesia. Normal saline (0.4 mL kg<sup>-1</sup> min<sup>-1</sup>) was administered to each dog via intravenous route with a relatively constant rate during the experiment. The calculated amount of epinephrine was added to the first 0.5l of saline. Urine was collected via urinary catheter at different time intervals (0-30, 30-90, 90-150 and 150-210 min). The volume of urine at each stage was measured and a 50 mL sample was acidified by adding small amount of hydrochloric acid in order to stabilize the metabolite. VMA contents of urine samples were assayed using Biosystems kits (Costa Brava 30, Barcelona, Spain) by concurrent use of chromatographic separation and spectrophotometric detection according to the modified Wybenga and Pileggi method<sup>[26]</sup>. Detection limit of the method was 1.9 mg L<sup>-1</sup> and coefficient of variation was less than 5%. Data were analyzed by independent Student's t-test.

## RESULTS

Table 1 shows a summary of the data obtained with respect to the effect of salinomycin on the urinary excretion of VMA in dogs. Table 2 shows the total amounts of VMA excreted in urine during various time intervals. Dogs in group I excreted an average of 196 µg of VMA in urine compared to 81 µg in group II during first half an h i.e., before intravenous infusion of adrenaline (Table 2).

Table 1: Summary of data obtained from determination of VMA excreted in urines of dogs at various time intervals following intravenous administration of adrenaline (10 µg kg<sup>-1</sup>)

Groups	I <sup>a</sup>					II <sup>b</sup>					
	1	2	3	4	5	6	7	8	9	10	
Dog No. →											
Body weight (kg) →	24	23	26	22	29	23	24	25	28	24	
Urine volume at various time intervals (mL)	0-30 <sup>c</sup>	85.0	100.0	50.0	75.0	85.0	110.0	95.0	95.0	70.0	90.0
	30-90	110.0	145.0	90.0	100.0	120.0	90.0	70.0	120.0	100.0	130.0
	90-150	45.0	90.0	140.0	140.0	90.0	120.0	105.0	80.0	95.0	65.0
	150-210	170.0	110.0	100.0	120.0	105.0	85.0	110.0	115.0	140.0	120.0
Urinary VMA concentration (µg mL <sup>-1</sup> )	0-30	2.71	1.69	1.69	3.49	2.75	0.56	1.18	0.75	1.07	0.94
	30-90	2.91	3.91	3.81	5.25	3.10	0.95	1.61	2.14	1.93	1.31
	90-150	3.47	4.03	4.22	8.57	3.28	1.06	2.36	1.50	2.14	1.60
	150-210	3.75	5.61	5.92	7.09	4.65	1.13	4.51	2.68	1.72	1.88
Total amount of VMA excreted at various time intervals (µg)	0-30	230.4	169.0	84.5	261.8	233.8	61.6	112.1	71.3	74.9	84.6
	30-90	320.1	567.0	342.9	525.0	372.0	85.5	112.7	256.8	193.0	170.3
	90-150	360.0	362.7	590.8	540.0	295.2	127.2	247.8	120.0	203.3	104.0
	150-210	637.5	617.1	592.0	850.8	488.3	96.1	496.1	308.2	240.8	225.6

<sup>a</sup>: Receiving adrenaline 150 min after oral whole egg; <sup>b</sup>: Receiving adrenaline 150 min after oral salinomycin (1 mg kg<sup>-1</sup>) mixed with whole egg; <sup>c</sup>: Min

Table 2: Total amounts ( $\mu\text{g}$ ) of VMA in urine samples collected from dogs following intravenous administration of adrenaline ( $10 \mu\text{g kg}^{-1}$ )

Time intervals (min)	Group I <sup>a</sup>	Group II <sup>b</sup>
0-30	195.9 $\pm$ 24.7	80.90 $\pm$ 6.2 <sup>S</sup>
30-90	425.6 $\pm$ 43.2	163.7 $\pm$ 23.1 <sup>S</sup>
90-150	429.7 $\pm$ 48.5	160.5 $\pm$ 23.3 <sup>S</sup>
150-210	637.1 $\pm$ 47.8	273.4 $\pm$ 46.1 <sup>S</sup>
Mean ( $\mu\text{g h}^{-1}$ )	497.5 $\pm$ 30.5	199.1 $\pm$ 23.0 <sup>S</sup>

<sup>a</sup>: Receiving adrenaline 150 minutes after oral whole egg; <sup>b</sup>: Receiving adrenaline 150 min after oral salinomycin ( $1 \text{ mg kg}^{-1}$ ) mixed with whole egg; <sup>S</sup>: Significantly different from corresponding value found in group I

The total amount of VMA excreted in urine during 30-90, 90-150 and 150-210 min after administration of adrenaline to dogs in group I were around 426, 430 and 437  $\mu\text{g}$ , respectively. The corresponding values in group II were found to be 164, 161 and 273  $\mu\text{g}$ , respectively. In summary, the average rate of VMA excretion were calculated to be around 498 and 199  $\mu\text{g h}^{-1}$  in group I and II, respectively (Table 2).

## DISCUSSION

From previous reports published by other researchers, it seems that the ionophores could alter contraction of the heart simply by releasing catecholamines<sup>[17,27,28]</sup>. Since the release of noreadrenaline from adrenergic nerve endings specifically requires calcium, the action of the antibiotics could involve an alteration in calcium permeability which then leads to a slow, modulated release of transmitter<sup>[17]</sup>. The attenuation of the inotropic effects produced by lasalocid and monensin by beta adrenergic blockers has been the basis of the conclusion that catecholamine release mediates in part the cardiotoxic effects of ionophores<sup>[15,17,28]</sup>.

In describing the cardiovascular impact of ionophores, researchers have highlighted the positive effect of these compounds on the release of noreadrenaline. For example, Suchard *et al.*<sup>[22]</sup> reported that ionophore monensin stimulates secretion of catecholamine from cultured chromaffin cells via a rise in intracellular sodium. In addition, Perlman *et al.*<sup>[21]</sup> have found that a number of carboxylic ionophores stimulate the secretion of noreadrenaline from cell suspensions prepared from a transplantable rat pheochromocytoma. They believed it is likely that ionomycin-induced catecholamine secretion results from the ionophore-mediated entry of  $\text{Ca}^{2+}$  into the cells. However, monovalent-cation ionophore monensin was supposed to stimulate catecholamine secretion by a mechanism that is independent of extracellular  $\text{Ca}^{2+}$ <sup>[21]</sup>. Moreover, Fahim *et al.*<sup>[18]</sup> reported that a major part of

inotropic effect of salinomycin on the dog heart may be attributed to the release of endogenous catecholamines. No direct analytical confirmation of this in the intact animal has, however, been reported, nor has any relation been established between plasma catecholamine concentrations and the cardiovascular effects produced by ionophores<sup>[18]</sup>. On the other hand, data obtained by Schwartz *et al.*<sup>[16]</sup> demonstrates that the ionophore lasalocid (Ro 2-2985) is not a noreadrenaline-releasing agent in a manner similar to tyramine, since even extremely large amounts of the drug do not cause a major demonstrable depletion of noreadrenaline.

It is possible that one of the actions of the ionophore antibiotics is to alter in some way the distribution of certain myocardial factors, one of which could be a histone. It should also be noted that small concentrations of histone produce a highly significant alteration in the inner membrane structure of mitochondria, affecting a passive release of potassium<sup>[16]</sup>.

Lasalocid can also form lipid-soluble complexes with catecholamines<sup>[29]</sup> and can transport catecholamines across artificial lipid membranes<sup>[30]</sup>. Triggle<sup>[31]</sup> has reviewed the subject of ionophore-induced catecholamine secretion and has proposed that lasalocid may promote catecholamine release from chromaffin cells and sympathetic neurons both by stimulating exocytosis and by acting directly as a catecholamine ionophore.

The study presented here is the first report proposing a different mechanism which is possibly involved in the cardiovascular effects of ionophores. The urinary excretion of VMA during 0-30 min in dogs receiving salinomycin were significantly ( $p < 0.05$ ) different from that in the control group (Table 2). In addition, dogs in group II (receiving adrenaline after salinomycin) significantly ( $p < 0.05$ ) excreted less VMA compared to the animals in group I (receiving adrenaline alone).

## CONCLUSION

It is generally concluded that the adrenergic effect of salinomycin may be elicited by a decrease in catecholamine catabolism. Of course, one can make other postulations to describe the involved mechanism of the findings of the present study. For example, salinomycin can compete with VMA to be excreted in urine. Therefore, further works such as measurement of catecholamine concentrations (both parent and metabolites) in serum and determination of other catecholamine metabolites in urine and serum should be

considered. These may help to more clarify the mechanism(s) involved in this drug interaction.

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