COMPARISON OF THE ACUTE TOXICITY OF SODIUM SALINOMYCIN IN SYNVERTAS AND SACOX PREPARATIONS IN CHICKENS

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Abstract

The study presents a median lethal dose (LD₅₀) of sodium salinomycin in Slovak anticoccidial preparation Synvertas and in German anticoccidial preparation Sacox 120 for 4-week-old chickens. On the basis of acute toxicity of sodium salinomycin, defined in the preliminary experiment, the LD₅₀ was determined in a group of 40 Ross I meat hybrid chickens of both sexes, weighing 1320-1820 g. Clinical symptoms of poisoning and patho-morphological changes in dead chickens were observed as well. LD₅₀ of sodium salinomycin in the respective preparations was calculated using double-dose interpolation method according to Roth (1962) on the basis of 24 h mortality. The LD₅₀ for sodium salinomycin in Synvertas was 107.5 mg.kg⁻¹ b.w. (1075 mg of preparation) and that in Sacox 100.0 mg.kg⁻¹ b.w. (833.3 mg of preparation). From the clinical signs of intoxication after lethal doses of 100 and 120 mg of sodium salinomycin per kg⁻¹ b.w. (1000 and 1200 mg of Synvertas preparation and 833.3 mg and 1000 mg of Sacox preparation), the following predominated: locomotor ataxia, semiprone or sternal lying position with neck stretched forward and legs stretched backwards, dyspnoea, cyanosis, serious central nervous system inhibition, loss of vocal and tactile responses. Death occurred within 17 h and was accompanied by mild myoclonus symptoms with backward stretched limbs. Hyperaemia of the liver, pancreas, spleen and kidneys, anaemia of GIT mucosa, diffuse acute bronchopneumonia (lung oedema in one chicken), slight anaemia of thoracic musculature and slight banding of thigh and thoracic musculature were observed in the majority of dead chickens. The toxic effect of sodium salinomycin started to manifest itself after a sublethal dose of 20 mg.kg⁻¹ b.w. (200 mg of Synvertas preparation and 166.6 mg.kg⁻¹ b.w. Sacox preparation). Poisoning symptoms were very weak and disappeared after 15 h.

Key words: chickens, sodium salinomycin, Synvertas, Sacox, toxicity, symptoms, pathology.

Effective prevention of coccidiosis is one of the basic preconditions of broiler production in large-scale operations. Along with observation of animal hygiene preventive principles, systematic application of anticoccidials in feed in compliance with rules of their safe application is a decisive and essential preventive factor against coccidiosis outbreaks. Due to the fact that the currently used ionophorous anticoccidials (monensin, narasin, salinomycin, lasalocid, maduramycin, semduramycin) have narrow therapeutic range and some of them are highly toxic to some animal species and age categories (2, 8, 18, 19, 22, 24, 27), high requirements on their safe application are entirely justified. The risk of possible acute or chronic intoxication is closely linked with this issue not only with regard to overdosing. In relation to this risk it is necessary to consider that the effectiveness and toxicity of an ionophorous anticoccidial do not depend only on pharmacological profile, their dosage, and functional condition of biological systems, but also on a range of other endogenous and exogenous factors, including therapeutic formulation (7, 8).

Equal quantities of effective ingredients present in generic preparations manufactured by different producers do not guarantee that the respective preparations are equally effective and tolerated (5, 30). Therefore it is inevitable to carry out obligatory testing of newly introduced and innovated preparations that can provide as complete knowledge as possible about their effectiveness and the extent of relevant toxicological risk. This is eventually one of the requirements for the registration of anticoccidials.

This study presents information about the extent of toxicological risk to 4-week-old chickens treated with sodium salinomycin associated with the anticoccidial Synvertas compared to Sacox 120, which served a standard. The aim of testing of sodium salinomycin in the respective preparations was to determine and compare its acute oral toxicity (LD₅₀), clinical symptomatology of poisoning after sublethal and lethal doses, remission of clinical signs of intoxication and pathomorphological changes in dead chickens.
Material and Methods

Characteristics of the preparations. Synvertas (*plv. ad us. vet.* (Biotika, a.s., Slovenská Lúčka, the Slovak Republic)) is a brownish powder with a distinctive odour. According to the producer’s data, it contains 10% of sodium salinomycin. Sacox 120 *gran. ad us. vet.* (Hoechst Roussel Vet., Germany) is a fine brownish granulate with a characteristic odour. According to the producer’s data, it contains 12% of sodium salinomycin.

Determination of acute oral toxicity. A median lethal dose (LD<sub>50</sub>) of sodium salinomycin in Synvertas and Sacox 120 was determined after a preceding preliminary experiment. The aim of the preliminary experiment carried out on thirty 4-week-old Ross I meat hybrid chickens of both sexes was to estimate the toxicity of sodium salinomycin in the respective preparations. The chickens were divided to equal 10 subgroups. Chickens from the subgroups 1-5 were administered sodium salinomycin in the form of Synvertas, while chickens from the subgroups 6-10 were administered the compound in the form of Sacox 120. Sodium salinomycin in Synvertas was administered at the following doses: 20 mg.kg<sup>-1</sup> b.w (200 mg of preparation) in the subgroup 1, 30 mg.kg<sup>-1</sup> b.w (300 mg of preparation) in the subgroup 2, 40 mg.kg<sup>-1</sup> b.w. (400 mg of preparation) in the subgroup 3, 50 mg.kg<sup>-1</sup> b.w. (500 mg of preparation) in the subgroup 4, and 70 mg.kg<sup>-1</sup> b.w. (700 mg of preparation) in the subgroup 5. Sodium salinomycin in Sacox 120 was administered as follows: 20 mg.kg<sup>-1</sup> b.w. (166 mg of preparation) in the subgroup 6, 30 mg.kg<sup>-1</sup> b.w. (250 mg of preparation) in the subgroup 7, 40 mg.kg<sup>-1</sup> b.w. (333.3 mg of preparation) in the subgroup 8, 50 mg.kg<sup>-1</sup> b.w. (416.6 mg of preparation) in the subgroup 9, and 70 mg.kg<sup>-1</sup> b.w. (583.3 mg of preparation) to the chickens in the subgroup 10.

The preparations were administered in the morning in the form of suspension (in water, at a ratio of 1:5) *per os* through a probe as a single dose. During the experiment the chickens were fed standard HYD-02 mixed feed. They had unlimited access to water. In the course of the preliminary experiment the chickens were checked for development and remission of clinical symptoms of intoxication at the following time intervals: 0.5, 1, 2, 3, 4, 5, 6, 9, 21, and 24 h, and twice a day during the subsequent 3 d.

Determination of LD<sub>50</sub>. After estimation of the acute toxicity of sodium salinomycin in the respective preparations, LD<sub>50</sub> was determined in a group of 40 chickens (4-week-old, weighing 1320-1820 g) of the same breed and sex using a double dose interpolation method (23) based on 24 h mortality. The chickens were fed complete mixed feed HYD-02, and had unlimited access to water. They were reared on deep litter at identical microclimatic conditions.

The chickens were divided into equal 4 subgroups. Chickens from the subgroups 1 and 2 were administered sodium salinomycin in the form of Synvertas and chickens from the subgroups 3 and 4 were administered the compound in the form of Sacox 120. Sodium salinomycin in the form of Synvertas was administered at the following doses: 100 mg.kg<sup>-1</sup> b.w. (1000 mg of Synvertas) in the subgroup 1 and 120 mg.kg<sup>-1</sup> b.w. (1200 mg of Synvertas) in the subgroup 2. Sodium salinomycin in the form of Sacox 120 was administered as follows: 100 mg.kg<sup>-1</sup> b.w. (833.3 mg of Sacox) in the subgroup 3 and 120 mg.kg<sup>-1</sup> b.w. (1000 mg of Sacox) in the subgroup 4.

Both preparations were administered *per os* by probe as a single dose in the morning, in the form of suspension (in water, at a ratio of 1:5). After administration of the drugs the chickens were observed for the development of clinical signs of poisoning and the time of death or remission of intoxication symptoms (surviving chickens). Clinical observations were carried out 30 min after administration of the drugs and then at 1 h intervals during the subsequent 12 h and after 24 h. Exited chickens were examined pathomorphologically.

Results

Acute oral toxicity. The LD<sub>50</sub> of sodium salinomycin in Sacox 120 preparation for 4-week-old chickens was 100.0 mg.kg<sup>-1</sup> b.w. (833.3 mg of preparation) with the upper limit of reliability 122.4 mg.kg<sup>-1</sup> b.w. (1020 mg of preparation) and the lower one 81.6 mg.kg<sup>-1</sup> b.w. (680.0 mg of preparation) of b.w. The LD<sub>50</sub> of sodium salinomycin in Synvertas for 4-week-old chickens was 107.5 mg.kg<sup>-1</sup> b.w. (1075 mg of preparation) with the upper limit of reliability 116.2 mg.kg<sup>-1</sup> b.w. (1162 mg of preparation) and the lower one 99.5 mg.kg<sup>-1</sup> b.w. (995 mg of preparation). LD<sub>50</sub> of sodium salinomycin and other relevant parameters are presented in Table 1.

<table>
<thead>
<tr>
<th>Dose of sodium salinomycin (mg.kg&lt;sup&gt;-1&lt;/sup&gt; b.w.)</th>
<th>Number of exited chickens up to 24 h / total</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (mg.kg&lt;sup&gt;-1&lt;/sup&gt; b.w.)</th>
<th>Limit of reliability (mg.kg&lt;sup&gt;-1&lt;/sup&gt; b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synvertas 100</td>
<td>5/10</td>
<td>100.0</td>
<td>81.6</td>
</tr>
<tr>
<td>120</td>
<td>8/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacox 100</td>
<td>3/10</td>
<td>107.5</td>
<td>99.5</td>
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Symptoms of intoxication. Graded doses of sodium salinomycin in the form of Synvertas and Sacox 120 administered per os in the preliminary experiment, resulted in the following symptoms. Lower doses (20, 30 and 40 mg.kg\(^{-1}\) b. w.) induced mild weakness of limbs, slightly drooping wings, mild incoordination, more frequent resting, unwillingness to move, mild dyspnoea and increased respiration rate, and loss of appetite. After administration of higher doses (50 and 70 mg.kg\(^{-1}\) b.w.) the symptoms were the same but more intense. The chickens were lying with stretched limbs and drooped wings and were unable to rise. A loss of vocal response was observed. Clinical symptoms of poisoning persisted for 1.5–2 h after lower doses and for 45–60 min after higher doses. The normal status was restored after 15 h with lower doses and after 2–3 d following the higher doses.

After administration of 100 and 120 mg of sodium salinomycin per kg\(^{-1}\) b. w. the intoxication symptoms developed within 30 min. Death occurred within 2–17 h after the administration. The following symptoms were observed: locomotor ataxia, semiprome of sternal lying position with neck stretched forward and legs stretched backward, dyspnoea, cyanosis, serious central nervous system disturbances and loss of vocal and tactile responses. Death was accompanied by mild myoclonus (3–4) with limbs stretched backward.

Administration of 100 mg.kg\(^{-1}\) b. w. of sodium salinomycin in the form of Sacox caused death of 5 out of 10 chickens while 3 out of 10 chickens died after Synvertas. The mortality after the dose of 120 mg.kg\(^{-1}\) b. w. was 8 out of 10 chickens with Sacox and 8 out of 10 with Synvertas.

Pathological and morphological changes. Hyperaemia of the liver, pancreas, spleen and kidneys, anaemia of gastrointestinal tract mucosa, diffuse acute catarhal bronchopneumonia (lung oedema in one chicken), mild anaemia of thoracic musculature, slight banding of thigh and thoracic muscles were observed in the majority of dead chickens.

Discussion

Since the discovery of the anticoccidial effect of ionophore antibiotic salinomycin (16) many data were reported about its toxicity, side effects, interactions, incompatibility, and residues (1, 3, 4, 11, 13, 15, 18, 19, 27). The highest tolerated concentrations of sodium salinomycin for individual animal species determined in Hoechst laboratory tests were reported by Plíšek (22).

The recommended dose of sodium salinomycin for chickens ranges from 40 to 60 mg.kg\(^{-1}\) (3, 4), and the maximum tolerated dose is 90 mg.kg\(^{-1}\) (11, 27). In other studies 60 mg.kg\(^{-1}\) and 120 mg.kg\(^{-1}\) of salinomycin were used as the therapeutic and toxic doses (9, 10). LD\(_{50}\) of sodium salinomycin (Hoechst) determined by Dost (3) is 44.5 mg.kg\(^{-1}\) b. w. in chickens, 57 mg.kg\(^{-1}\) b. w. in mice, 50.3 mg.kg\(^{-1}\) b. w. in rats and 21 mg.kg\(^{-1}\) b. w. in rabbits. LD\(_{50}\) of sodium salinomycin (Pfizer) in rats is 50 mg.kg\(^{-1}\) b. w. (28). Because there is no guarantee that generic preparations (preparations with identical active ingredient and identical dosage) manufactured by various producers are equivalent in their effect and toxicity (5, 6, 29, 30) all newly introduced and innovated preparations must be tested not only for their basic pharmacological parameters, acute toxicity, but also for safety of recommended dosage and side effects associated with repeated or continuous administration of higher than recommended doses. Keeping this in mind we decided to determine the LD\(_{50}\) value of sodium salinomycin in the preparation Synvertas of Slovak provenience and compare it with that of the preparation Sacox, produced in Germany, which served as a standard. The LD\(_{50}\) of sodium salinomycin for 4-week-old chickens determined in our study was 107.5 mg.kg\(^{-1}\) b.w. when administered in Synvertas and 100.0 mg.kg\(^{-1}\) b.w. in Sacox. Our observations indicated that the Synvertas was relatively less toxic to 4-week-old chickens than the foreign Sacox. The relatively low level of toxicity to chickens of sodium salinomycin in Synvertas was indicated by our results obtained under the conditions of acute (18) and subacute toxicity (19). This statement is supported by our findings in 3-day-old chickens fed Synvertas and Sacox for 42 d (27). From the possible causes of relatively lower toxicity of sodium salinomycin in Synvertas compared to Sacox not only different additive substances should be considered but also the factor of higher concentration of sodium salinomycin in Sacox. Higher concentration of the active ingredient induces usually a more complex and more rapid resorption. However, it is necessary to take into account different methods of calculating of LD\(_{50}\) used in the determination of acute toxicity as well as the fact that the LD\(_{50}\) value may be affected by selection of experimental animals (age, sex, breed, present health condition) or the choice of vehicle.

Observation of the poisoning symptoms indicated that the toxic effect of sodium salinomycin began to manifest itself at a sublethal dose of 20 mg.kg\(^{-1}\) b. w. However, the intoxication symptoms were very mild. The fact that they disappeared within 15 h after administration suggested its relatively short biological half-time. The abnormal position of head and limbs, limb paralysis, serious CNS inhibition, dyspnoea, loss of vocal and tactile responses after lethal doses seemed to be the dominant symptoms of poisoning. Intoxication symptoms observed in our study corresponded more or less to the poisoning symptoms presented by Mazlum (14) in chickens or Šály (26), Neufeld (17), and Novilla (20) in turkey hens.

Although the presented autopsy findings (hyperaemia of the liver, pancreas, spleen, kidneys, anaemia of GIT mucosa, acute catarhal pneumonia, lung oedema in one chicken, slight banding of thigh and thoracic muscles) were common to the majority of dead birds, a portion of chickens showed only some of the changes. The macroscopic changes observed in our study corresponded to pathomorphological changes presented by Šály (26) and Yong (31) in turkey hens. No acute bronchopneumonia was reported as one of autopsy findings.
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References