

A SPECIAL CASE OF AN ACUTE TIAMULIN-SALINOMYCIN INTOXICATION IN PIGS DUE TO RESIDUAL TIAMULIN FOUR MONTHS AFTER MEDICATION

Een bijzonder geval van een acute tiamuline – salinomycine intoxicatie bij varkens door drinkwaterresidus vier maanden na medicatie

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ABSTRACT

An acute salinomycin poisoning was diagnosed in a pig farm, in this case in one compartment housing 200 weaning piglets. The poisoning was caused by a drug incompatibility due to the administration of salinomycin in the feed and the presence of traces of tiamulin left in the drinking water, resulting from the fact that tiamulin had been administered to fattening pigs housed in the stable four months before the appearance of the clinical symptoms in the piglets. The main symptoms of poisoning were loss of appetite and locomotor disturbances. The locomotor symptoms consisted of weakness of the posterior body half and lateral decubitus in more severe cases. Finally, 35 of the 200 piglets died. Histological study of the skeletal muscles, biochemical study of the blood, and chemical analysis of the drinking water for tiamulin were performed to establish the diagnosis.

SAMENVATTING

Een salinomycine-intoxicatie werd vastgesteld in een gesloten varkensbedrijf, namelijk in één afdeling met 200 gespeende biggen. De intoxicatie werd veroorzaakt door het toedienen van salinomycine in het voeder in aanwezigheid van resterend tiamuline in het drinkwater, die merkwaardig genoeg nog afkomstig was van een vier maanden eerder toegediende medicatie. De voornaamste symptomen van intoxicatie waren anorexie en bewegingsstoornissen, voornamelijk ataxie van de achterhand, abnormale stand van de achterpoten (overkoot staan) en bij enkele biggen laterale decubitus. Uiteindelijk stierven 35 van de 200 biggen. Histologisch onderzoek van de skeletspieren, biochemisch onderzoek van het bloed en chemische analyse van het drinkwater voor tiamuline werden uitgevoerd om de diagnose te stellen.

Keywords: Tiamulin-Salinomycin intoxication - Pig

INTRODUCTION

It is well known from the literature that tiamulin may interact with polyether ionophorous antibiotics such as monensin, narasin and salinomycin (Anadón and Martínez-Larrañaga, 1991, Drake, 1981, Horrox, 1980, Miller *et al.*, 1986). Tiamulin is a derivative of pleuromutulin, an active antibiotic obtained from the

mould *Pleurotus mutilis*. It is used in pigs and poultry for the treatment of respiratory infections and in pigs for the control of swine dysentery. The combining of tiamulin with ionophorous antibiotics produces primary ionophore myotoxicity in chickens, turkeys and pigs (Osweiler, 1996). Toxicosis by salinomycin and other ionophores in animals is potentiated by drugs

that inhibit specific pathways in hepatic metabolism (Meingassner *et al.*, 1979). Tiamulin interacts with the biotransformation of these ionophores, decreasing ionophore clearance. However, Dost (1991) showed that simultaneous treatment with salinomycin and tiamulin is possible without any risk for incompatibility, provided the sum of the dosages of both products is below 6 mg/kg BW. Calculations based on average food and drinking water intakes result in a sum total of 100 mg/kg feed that can be considered safe, (e.g. 20 mg/kg salinomycin in the feed can be combined with 80 mg/kg tiamulin in the feed). This dosage of 6 mg tiamulin + salinomycin/kg BW has recently been a topic of dispute. Wendt *et al.* (1997) found clinical signs of poisoning for dosages of 8, 6 and 4 mg tiamulin + salinomycin/kg BW.

The purpose of this report was to provide evidence for a special case of a (delayed) tiamulin mediated salinomycin intoxication in piglets.

CASE HISTORY

Two hundred sows and 1600 fattening pigs were housed in a pig farm. The following treatment was performed for a compartment of 200 piglets (weaned about three weeks earlier).

A four-day treatment with organic acids was done to control an *Escherichia coli* diarrhea, in this case with 1.5 l/1000 l Selko-pH organic acids (Selko B.V., Tilburg, The Netherlands). This mixture contained ammonium formate, formic acid, acetic acid, citric acid, ascorbic acid and copper acetate (0.4%). Accord-

ing to the manufacturer, this solution lowers the pH of the drinking water to a pH of about 4. During this treatment, the piglets received a commercial weaning feed containing 60 mg/kg salinomycin.

Three days after the start of the Selko-pH treatment, anorexia was observed in 75% of the animals and some piglets were already displaying lateral decubitus. Five piglets showed motor disturbances with weakness of the posterior body half, inability to stand and abnormal position of the posterior legs. Two days after the onset of the first symptoms, the administration of the Selko-pH organic acids was stopped and the medicated drinking water circuit was switched to another (separate) circuit containing only tap water. Thereafter, additional piglets suffering from anorexia and motor disturbances were observed. Treatment with ceftiofur did not improve the health status of the affected animals. As the piglets were receiving salinomycin in their feed, the concentration of salinomycin in the feed was checked and confirmed to be correct by the manufacturing company. Only after the salinomycin feed medication was stopped did the progressive recovery of the majority of the animals begin. The situation was completely normalized 12 days after the salinomycin feed medication was stopped. In the end, 35 out of the 200 piglets died. Table 1 summarizes the treatments performed and the symptomatology observed in the intoxicated piglets.

A history check on the farm revealed that previously housed fattening pigs had been treated with tiamulin to control an outbreak of swine dysentery caused by *Brachyspira hyodysenteriae*. This treatment had

Table 1. Summary of the treatments administered to the 200 piglets and the symptomatology seen in piglets suffering from a tiamulin-salinomycin intoxication.

Time (days)	
1	Start of the Selko-pH organic acids administration in the drinking water
4	First symptomatology: anorexia in 75 % of the animals and motor disturbances in 5 piglets
5	Stop of the Selko-pH organic acids administration and provision of tap water
6 → 11	Additional intoxicated piglets and death of 35 animals
12	Stop of the salinomycin feed medication
13 → 24	Progressive recovery
25	Normalized situation

been going on for about four months before the onset of the clinical symptoms in the piglets. Two tiamulin formulas were administered. First, tiamulin (Tiamutin Premix 10 %) was administered for three weeks via the feed (V.M.D., Arendonk, Belgium). Thereafter, treatment was started with tiamulin (Tiamutin Pigs 45 %, V.M.D.) via the drinking water. This tiamulin powder was added to the same water tank that the Selko-pH solution was added to. Medicated drinking water was prepared every day for a total of 12 days, each time in a dosage of 80 g Tiamutin Pigs/1000 l water. In total, 1 kg of Tiamutin Pigs or 450 g tiamulin was added to the drinking water tank.

The medicated drinking water system consisted of a plastic water tank of about 1000 l, connected via plastic piping to drinking nipples. Medicated water was pumped at a pressure of about 2 bar, which provided a slight stirring movement in the tank. The volume in the tank was kept constant at about 100 l by means of a bottom floating gauge, until another addition of 80 g Tiamutin Pigs to 1000 l was performed. This remaining 100 l of medicated drinking water could not be removed due to the construction of the tank. After medication, the drinking water circuit was switched to a circuit without medication.

It appeared that between the tiamulin treatment and the administration of the Selko-pH, other treatments using the same water tank had been given to the previously housed fattening pigs. These included a treatment with lincomycin (Lincocin 40 %, Pharmacia & Upjohn, Puurs, Belgium), which was administered 7 times to 1000 l of water, and three administrations of pyrantel (Banminth, Pfizer Animal Health, Nossesgem, Belgium), also added to 1000 l of water. These drugs were administered without causing any adverse reactions.

When the intoxicated piglets died, post-mortem histological examinations were performed. Blood from some piglets was taken for biochemical examination. Moreover, drinking water that was sampled at the moment of the administration of the Selko-pH organic acids was sent for laboratory analysis to quantify tiamulin. Samples of the sediment on the walls and bottom of the water tank were also analysed for tiamulin. An aliquot of the sediment on the bottom of the tank was also mixed with Selko-pH organic acids to improve the solubility of possible residual tiamulin.

LABORATORY EXAMINATIONS

Histology and biochemistry

The histopathological examinations of the skeletal muscles revealed a hyaline degeneration of the muscle cells. A diffused presence of myocytes with a hyaline aspect was observed, along with rounding and centralizing of the nuclei with loss of striation.

The activities of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) in the serum were evaluated and showed very high levels, in this case 10,840 mU/ml LDH and 115,200 mU/ml CPK. Normal levels of LDH in pigs range between 40 and 700 mU/ml. CPK levels below 200 mU/ml are reported as normal. These results indicate massive muscle cell degeneration.

Tiamulin quantification

The tiamulin concentration in drinking water and in sediment on the walls and bottom of the drinking water tank was determined using a high-performance liquid chromatography (HPLC) method with diode-array UV detection at $\lambda=250$ nm. External standardisation was used to quantify tiamulin. One hundred μ l of the medicated drinking water was injected directly into the HPLC ($n=3$). One gram of the sediment on the walls and bottom of the tank was mixed with 2 ml of tap water and centrifuged at 3,600 rpm. Again, 100 μ l of the supernatant was injected. Detection was performed using a diode-array UV detector, monitoring between 200 and 325 nm. The limit of detection (LOD) of the analytical method, defined as the signal that corresponds to three times the noise, was 8.7 mg tiamulin/l water.

Table 2 shows the results of the tiamulin quantification. Tiamulin could only be detected in the drinking water and not in the sediments on the walls and bottom of the water tank. Even after the mixing of the sediment on the bottom of the tank with Selko-pH organic acids, tiamulin concentrations were below the LOD. The apparent tiamulin peak in the chromatogram was checked for identity by comparison with the UV spectrum of the compound in a standard sample and in the drinking water sample (200–325 nm). The UV spectra were identical, so the identity of tiamulin was confirmed.

Table 2. Concentration of tiamulin in the drinking water and in the sediment on the walls and bottom of the water tank used for medication.

Sample identification	Tiamulin concentration
1	148 mg/l \pm 8.1 mg/l (n=3)
2	< LOD*
3	< LOD (n=2)
4	< LOD (n=2)

* <LOD: value below the LOD of 8.7 mg/l water

1. Drinking water medicated with Selko-pH organic acids.
2. Sediment on the walls of the tank, taken one week after the end of the administration of Selko-pH organic acids.
3. Sediment on the bottom of the tank, taken three weeks after the end of the administration of Selko-pH organic acids.
4. Sediment on the bottom of the tank (cfr. sample 3), but mixed with Selko-pH organic acids.

DISCUSSION

In this case, both the symptoms and the histological and biochemical examinations already indicated a polyether antibiotic intoxication. Ionophores alter the transport of sodium, potassium or calcium, thus causing toxicologic damage, especially to the musculoskeletal system, which leads to mitochondrial calcium overload, impaired oxidative phosphorylation and cellular necrosis (Osweiler, 1996). Ionophores are known to have a narrow range of safety and are generally very toxic if an overdose is given (McDougald and Roberson, 1988). However, the feed salinomycin concentrations were within the dosage limits. The anamnesis revealed a tiamulin mediated salinomycin intoxication, which was confirmed by chemical analysis of the drinking water for tiamulin. It is remarkable, however, that the clinical symptoms of the salinomycin intoxication were only seen four months after the administration of tiamulin in the drinking water. It is striking that this moment coincided with the administration of a mixture of organic acids for a diarrhea treatment. One possible explanation is based on the higher solubility of residual tiamulin at lower pH values. Although treatments with Lincocin 40 % and

Banminth had already been performed between the tiamulin treatment and the administration of Selko-pH, the solubility of tiamulin seemed to be highly improved due to the lowering of the pH of the medicated water, provoked by the administration of organic acids.

It is very surprising, however, to see that tiamulin was still present at high concentrations in the tank, even four months after the last administration of the antibiotic and after several intermediate treatments with other drugs. Data from the manufacturing company revealed solubility problems with tiamulin at pH values of 7 or higher, but only with concentrations higher or equal to 1 % of the commercial Tiamutin Pigs formulation (L. Aerden, personal communication, 2000). These findings seem not to be in accordance with our results, since only 0.008% was added to the tank upon each refill. It is likely that other factors are of greater importance in this case. Here we are thinking of the failure to clean the drinking water tank after the medication was stopped and the failure to prepare a pre-solution of the Tiamutin Pigs formulation. Investigations revealed that the Tiamutin was prepared with a pre-solution only six out of the 12 times it was administered. The other six times, the tiamulin powder was put directly into the tank and it is likely that the powder did not dissolve completely in the drinking water, but rather formed a deposit on the bottom of the tank. The addition of the Selko-pH organic acids increased the solubility of the tiamulin sediment.

Concerning the mechanism of this chemical interaction, it is known that tiamulin inhibits the oxidative drug metabolism of certain compounds (such as the polyether ionophorous antibiotics) by the formation of a cytochrome P450 metabolic intermediate complex (Witkamp *et al.*, 1994, 1995, 1996). The results of these studies suggest a selective inhibition of cytochrome P450 isoenzyme(s) by tiamulin in rats and pigs, probably belonging to the CYP3A subfamily. Subsequently, after a certain lag-time, the total body clearance of salinomycin, which is metabolised by the same cytochrome P450 enzymes, is decreased and the elimination half-life is increased significantly in tiamulin-treated pigs (Anadón and Reeve-Johnson, 1999). Accumulation of the antibiotic is therefore likely, certainly when the drug is continuously administered by feed medication. This also explains why the recovery of the piglets started only when the salinomycin feed medication was stopped.

In conclusion, on the basis of this case we can state that the following of the GVP guidelines for the pre-

paration of pre-solutions intended for drinking water medication and good maintenance and cleaning of a medicated drinking water circuit can be of great importance for correct and safe treatment.

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