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GENOTOXICITY OF THE ANTICOCCIDIAL AGENT SALINOMYCIN

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This report presents an analysis of genotoxic effects of the ionophoric antibiotic salinomycin commonly used in the control of coccidiosis. Mutagenic properties were determined, after application of increasing doses to experimental chinchilla rabbits, by monitoring mitotic activity of bone marrow cells, numerical chromosomal alterations and structural chromosomal aberrations. Moderate to non-significant genotoxicity was observed, except for the frequency of structural chromosomal aberrations where 19% of the examined mitoses displayed aberrations. This could lead to sterility or termination of embryonic development and, therefore, calls for caution in its use in clinical practice and poultry farming.

Key Words: Genotoxicity, mutagenicity, chromosomal aberrations, anticoccidials, salinomycin

INTRODUCTION

Toxicology has two goals. The first is to identify and characterize the adverse effects that can be produced in biological systems by exposure to chemicals and the second is to use this information to predict the type and severity of responses in other species and exposure situations. The tools that the toxicologist uses to detect and describe the adverse effects of chemical exposure include the traditional acute, subchronic, and chronic studies in animals plus a variety of special studies designated to demonstrate specific organ damage, reproductive and teratogenic effects, neurotoxicity, immunotoxicity, genotoxicity, and other responses(Doull, 1996).

When the prime target of xenobiotics is the genetic material, toxicology evolves into genotoxicology, already a scientific discipline of its own. The importance of this field of biomedical science is well documented and amply reviewed

(Shimada, 1996, Sofini, 1966, Bridge, 1996). The reliability of some well established diagnostic methods for determination of mutagenicity of chemicals has been questioned and shown to yield contradictory results (Yoshikawa, 1996). For this reason test systems are continuously being improved upon and their scope extended (Josephy et al.,1997; Mark et. al., 1997, The greatest of all projects in the history of biomedical science, the human genome project, exerts its power, not unexpectedly, in the field of genotoxicology (Vorce and Stemmer, 1996). New technologies added new horizons to the study of mutagenicity of xenobiotics. This mainly refers to genetically engineered animals, either transgenic or "knock-out", for *in vivo* testing (Suter et al., 1996; Gorelick et al., 1997. Suzuki et al., 1997), and to a variety of applications based on the polymerase chain reaction in generalized mutation detection (Olsen et al., 1996). In spite of impressive technological advances, conventional approaches remain important in risk assessment of potentially mutagenic xenobiotics. Numerous reports ilustrate genotoxicity testing of chemicals that frequently affect humans and animals. Some recent examples include the mutagenic analysis of pesticides (Ruiz and Marzin, 1997; Bianchi-Santamaria et al., 1997), herbicides(Scasselati-Sforzolini et al., 1997; Gebel et al., 1997), insecticides (Amer et al., 1996), estrogen based drugs (Hundal et al., 1997), food additives (Matsui et al. 1996), antibiotics (Chetelat et al, 1996), virostatics (Thust et al., 1996) and some old psychotropic agents falsely declared as nonmutagenic (Gocke, 1996).

This report addresses the question of undesirable side effects of an important anticoccidial agent with respect to its potential genotoxic activity. The analyzed substance was the sodium salt of salinomycin widely used under the trade name Sacox. Cytogenetic analysis suggests that it induces minor abnormalities of the chromosomal complement. However, these alterations might suffice to lead to an unbalanced genome with possible reproductive abnormalities. This could lead to sterility, termination of embryonic development and, therefore, calls for caution in its use in clinical practice and poultry farming.

MATERIALS AND METHODS

Animals and treatment. Chinchilla rabbits were kept under standard laboratory conditions and treated with SACOX (Hoechst, Veterinar GmbH, Germany). The active substance of this anticoccidial, an ionophoric antibiotic, is the sodium salt of salinomycin. The treatment was designed to mimic farming conditions as realistically as possible. An adequate amount of briquetted, concentrated feed was evenly distributed over a plastic foil. The specified amount of the anticoccidial, finely suspended in water was uniformly dispersed over the feed. After thorough mixing, it was dried in air for two days. We believe that this preparation procedure ensures more reliable dosing than that under common

farming conditions. The experimental rabbits were six months old and weighed 2.5 - 3 kg. Both control and experimental groups contained seven animals each. Salinomycin was applied in doses of 50, 100 and 150 ppm, representing prophylactic, double prophylactic and triple prophylactic doses. The treatment lasted 30 days, when the animals were sacrificed and bone marrow prepared for cytogenetic analysis.

Bone marrow preparation and chromosome analysis. Mutagenicity of the anticoccidial agent SACOX was tested by monitoring chromosomal alterations in the bone marrow of treated animals. The marrow was prepared by a modification of a previously published protocol (Hsu and Patton, 1969). The preparations were stained in the conventional manner (5% Giemsa in phosphate buffer, pH 6.8).

Statistical analysis. Data were presented as averages, percentages, standard deviations and the statistical significance determined by the t-test.

RESULTS

Table 1 illustrates numerical values obtained as the consequence of the treatment with different dosages of Sacox. Average values of the mitotic activity of the control group and the experimental group under treatment with the prophylactic dose of Sacox, were identical.

Table 1. Mitotic activity following the treatment with Sacox

	$\bar{X}$	SD	Minimum – maximum	Statistical significance	Number of examined cells
K	4.4	0.8	3 – 6	–	1000
P	4.4	1.1	3 – 6	NS	1000
2P	3.8	0.7	3 – 5	NS	900
3P	3.1	0.8	2 – 4	$P < 0.05$	900

K = Control; P = Prophylactic dose; 2P = Double Prophylactic dose; 3P = Triple prophylatic dose

This suggests that this dose does not affect the proliferative activity of bone marrow cells. The application of double the prophylactic dose reduced the mitotic activity to an extent that was not statistically significant. Consequently, this dose range has no effect on mitotic activity of bone marrow cells. The triple prophylactic dose notably reduced the mitotic activity to a statistically significant 30% of the control values. In conclusion, there is an evident dose-dependent reduction of mitotic activity of bone marrow cells with acceptably low standard deviations.

The same set of experiments demonstrated numerical chromosomal alterations (Table 2).

Table 2. Numerical chromosomal alteration induced by Sacox

	< 2n (%)	2n (%)	> 2n (%)	Polyploidy	Number of examined mitoses
K	2 (1.9%)	99 (95%)	2 (1.9%)	1 (0.9%)	104
P	3 (2.7%)	105 (93.8%)	3 (2.7%)	1 (0.9%)	112
2P	4 (4.2%)	85 (88.5%)	5 (5.2%)	2 (2.1%)	96
3P	4 (5.1%)	68 (87%)	4 (5.1%)	2 (2.6%)	78

K = Control; P = Prophylactic dose; 2P = Double Prophylactic dose; 3P = Triple prophylactic dose

The frequency of mitosis with an abnormal number of chromosomes increased as the dosage of Sacox increased. Aneuploidies were a more frequent anomaly than polyploidies. Within aneuploidies, there was a roughly equal proportion of hypoploidies and hyperploidies. The total of their frequencies increased from 3.8% in control animals, through 5,4% in animals treated with the prophylactic dose, up to 9.4% in animals treated with double the prophylactic dose and 10.2% in animals that received triple the prophylactic dose. The increase was not as large as was the case with antineoplastics (Bradley et al., 1979; Soldatović et al., 1979; Ohtsuru et al, 1980, Zimonjić, 1982;) but significant and clearly dose dependent. For example, the number of mitoses with a chromosomal number lower than normal (2n) increased from 1.9% in the control group to 5.1% in animals treated with the triple prophylactic dose. Polyploidy was not affected by the application of the prophylactic dose while the double and triple dose caused two-fold and three-fold increases, respectively.

Structural chromosomal aberrations are illustrated in Table 3 and shown in Figure 1.

Table 3. Structural chromosomal alterations induced by Sacox

	Number of examined mitoses	Gap (%)	Structural alterations
K	69	2 (2.9%)	–
P	66	1 (1.5%)	–
2P	67	2 (2.9%)	1 (1.5%)
3P	66	7 (10%)	6 (9.1%)

K = Control; P = Prophylactic dose; 2P = Double Prophylactic dose; 3P = Triple prophylactic dose

Among structural abnormalities gaps and deletions prevailed while Robertson's fusions were rare. Gap type abnormalities were detected in 2.9% of the examined mitoses in the control group, while the triple prophylactic dose increased this frequency to 10,6%. Structural aberrations other than gap were undetectable in animals under the prophylactic treatment and in the control group. The value was 9.1% with the highest applied dose. The total frequency of

mitoses with abnormal chromosomes after the application of 150 ppm of Sacox reached 19.7%. Dicentrics, rings and minutes were not detected. (Figure 1)

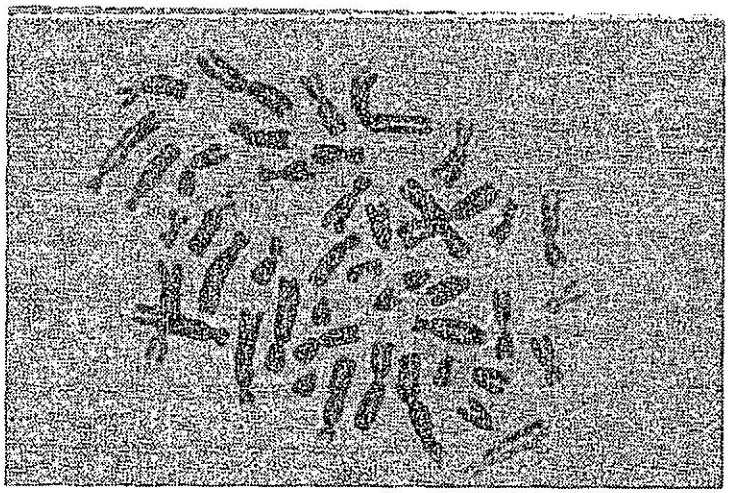

Figure 1. Structural aberrations (gaps) on the long arm of subacrocentric chromosome. The arrow indicates the gap.

DISCUSSION

Prophylaxis and therapy is no longer a matter of the interaction between a chemotherapeutic agent and an invading parasite. It has become axiomatic that the adverse effects of a beneficial drug must be analyzed for side effects that affect the host organism, and consequently, the outcome of the whole treatment procedure. This is particularly the case with protocols that require life-long duration. Prophylaxis of chicken coccidiosis is the archetype of this kind of problem. With these prospects in mind, the analysis of side effects of salinomycin, a widely used ionophoric antibiotic in poultry farming, is justified both from the scientific point of view, and as a source of potentially useful recommendations for veterinary practice.

We foused our research on the genotoxic effects of salinomycin fully aware that it is one of many consequences it may elicit in a host organism (Dimitrijević et. al. 1992). The scope of the experimental design was limited to an investigation of alterations in mitotic activity, numerical chromosomal anomalies and their structural aberrations. Salinomycin affects mitotic activity of bone marrow cells of

treated animals only to a small extent. This side activity of the drug is evident only at the triple prophylactic dose. Notably, this suppression is lower than that of cytostatics (Zimonjić, 1982) and similar to that of surfactants commonly used for disinfection (Vučinić,1992). These findings must be considered in relation to the fact that there need not be a correlation between the suppression of mitotic activity and the mutagenic potential of the substance under investigation (McFee, 1989). Deceleration, suppression or blockade of mitotic activity could be a consequence of different biochemical activities and it is more likely to be a plain cytotoxic effect rather than a mutagenic effect. Induced alterations in the number of chromosomes is notable but, again, lower than that of mutagenic drugs exemplified by cytostatics and without any similanity to that of surfactants. After the application of the maximal dose salinomycin causes 13% of the examined mitises to present with an altered chromosome number. For the sake of comparison, Mitomycin C, induces64% of mitoses with abnormal chromosome number in human lymphocyte cultures. Importantly, polyploidies never exceed 3%,which is below the threshold of statistical significance. This value classifies salinomycin as an agent that is not a serious risk for induction of an abnormal number of chromosomes. Structural chomosomal aberrations are a decisive experimental parameter in this type of analysis of genotoxicity of xenobiotics. They strictly define the level of risk for genome integrity incurred by the application of a drug. This equally holds for treated animals, personel engaged in the production and application of these drugs and for the human population consuming treated animals. The results obtained clearly indicate that the coccidiostat elicits significant structural alterations following the application of the maximal, tripleprophylactic dose, amounting to 19% of the examined mitoses. In this respect, it is quite comparable with typical mutagenic drugs in wide use, such as MitomycinC, Methotrexate and Endoxane and related compounds (Bradley et al., 1979; Soldatović et al., 1979; Ohtsuru et al., 1980; Zimonjić 1982; Martin, et al., 1997). Interestingly, our results are in accordance with the mutagenicity of some common anti-protozoal agents (Re et al, 1997). Lower doses induce structural aberrations at the very limit of statistical significance and can be considered safe from the point of view of the above mentioned risk groups.

All described results suggest that salinomycin induces minor defects in the chromosome complement but sufficient to cause an unbalanced genome. The consequences would be anomalous reproduction, sterility, termination of embryologic development, or anomalous offspring characterized by avitality and premature death. The majority of karyotypic alterations if balanced, will not be phenotypically evident in an affected individual, but may result in disordered gametogenesis resulting in a zygote with an unbalanced genome. One should

remember that under standard farming conditions animals take food and water *ad libitum*, and a triple prophylactic dose might not be a rare event. Taken together, these findings call for caution in its use in clinical practice and poultry farming.

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GENOTOKSIČNOST ANTIKOKCIDIJALNOG AGENSA SALINOMICINA

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SADRŽAJ

Predstavljena je analiza genotoksičnih efekata jonofornog antibiotika salinomicina koji se često koristi u suzbijanju kokcidioze. Mutagene osobine su ispitivane nakon aplikacije rastućih doza eksperimentalnim zečevima rase Činčila. Merena je mitotska aktivnost ćelija kostne srži, numeričke hromozomske aberacije i strukturne hromozomske aberacije. Uočena je umerena i nesignifikantna genotoksičnost izuzev u slučaju strukturnih hromozomskih aberacija gde je nađeno da 19% pregledanih mitoza ispoljava ove strukturne anomalije. Ovi poremećaji mogu biti uzrok pojave steriliteta ili prekida embrionalnog razvića, pa stoga, ukazuju na neophodnost pažljivog doziranja u kliničkoj praksi i uzgoju živine.