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Preliminary study on *in vivo* toxicity of monensin, salinomycin and their metal complexes

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The acute toxicity of the polyether ionophores monensin, salinomycin and their metal complexes with Na(I), Mg(II), Ca(II), Mn(II), Co(II), Zn(II) was evaluated in mice. The experimental data revealed that Ca(II) and Mg(II) complexes of salinomycin display the highest toxicity among the compounds tested, with LD_{50} values of 20.5 mg/kg b.w. (13 μmol/kg b.w.) and 25.8 mg/kg b.w. (17 μmol/kg b.w.), respectively. The preliminary evaluation of biochemical indices of survived animals showed that no significant changes occur within a three-day treatment with ionophorous antibiotics and their complexes.

Keywords: polyether ionophores, metal complexes, acute toxicity, biochemical indices

INTRODUCTION

Polyether ionophores are natural compounds produced by *Streptomyces spp*. and are applied in veterinary medicine as coccidiostats, antimicrobial agents and growth promoters [1]. Although known as monovalent ionophores for their affinity to bind alkali cations, these compounds also form various divalent metal derivatives depending both on the antibiotic form (acidic or sodium) and on the nature of the metal(II) ion [2-9].

Generally, the metal complexes of monensin and salinomycin possess more pronounced biological activity than non-coordinated ionophores as the antibacterial (*B. subtilis, B. mycoides, M. luteus*) and the anticancer studies (human squamous cell carcinoma, glioblastoma multiforme, cancers of lung, breast, liver and uterine cervix, chronic myeloid leukemia) revealed [9-12]. At the same time there are limited data on the toxicity of the polyether ionophorous antibiotics and especially of their metal-containing compounds in animal models [13, 14]. The aim of the present research is to evaluate both the acute toxicity of metal complexes of monensin and salinomycin in mice and their effect on some clinical parameters of survived animals.

EXPERIMENTAL

The protocol was approved by the Institutional Animal Care and Use Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences (IN-BAS) according to the Guidelines for Animal Experimentation.

Test compounds

Sodium forms of monensin and salinomycin were kindly provided by Biovet Ltd. Peshtera, Bulgaria. Acidic forms of antibiotics and their metal(II) complexes were prepared as previously described [2,3,5,6,9].

Animals and house conditions

Male ICR mice (18-25 g) were used in the experiments, housed in plastic cages with stainless steel top in the animal care facility of IN-BAS, where room temperature, humidity and ventilation were controlled according to international standards. The animals had access to food and water *ad libitum* and were maintained at 24 ± 2 ºC with a 12 h light/dark cycle.

Experimental design

The compounds tested were administered *per os* (gavage) on an empty stomach (12 h without food before testing) as aqueous suspensions. The acute

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toxicity (LD_{50}, mg/kg b.w., μmol/kg b.w.) was measured by the Prozorovsky's method [15].

The animals survived after acute toxicity test with the selected substances were subjected to additional two-day treatment with the corresponding dose of the compounds (total 72 h treatment). After that the animals were sacrificed under anesthesia (chloralhydrate, 50 mg/kg, *i.p.*). Blood samples were collected by cardiac puncture.

Clinical observations

The observation period was one or three days post administration for acute toxicity assay and 72 h treatment, respectively. Clinical signs of toxidromes (tremors, excitability, salivation, etc.) and mortality were observed while dosing, during the first two hours after administration and on the 12^{th} / 24^{th} h after the treatment.

Biochemical analyses

Biochemical examinations were performed using blood collected in plain tubes. Blood samples were centrifuged (3 000 rpm / 5 min) and the serum was collected for assays. The following parameters were measured on a Mindray clinical chemistry analyzer (China) using Sentinel (Italy) diagnostic kits: albumin (ALB), total protein (TP), aspartate aminotransferase (AST), alanin aminotransferase (ALT), alkaline phosphatase (ALP), lactatdehydrogenase (LDH), creatinine (CR), urea (URE).

Statistical analysis

The reference biochemical values of control animals were calculated by the parametric method [16]. The values of biochemical parameters do not usually follow the normal distribution and for that reason the reference limits are calculated using 2.5 and 97.5 percentile ranges.

RESULTS

The majority of acute toxicity tests performed aims to determine only the minimum lethal or maximum non-lethal dose. At the same time these tests can provide preliminary but useful information on the toxic nature of compounds for which no toxicological information is available. Such data base can be used to deal with cases of accidental ingestion of a large amount of the corresponding compound; to determine possible target organs and/or special tests that should be conducted in repeated-dose toxicity tests; and to select doses for short-term and sub-chronic toxicity tests when no other toxicology information is existing [17].

Monensin and salinomycin are well known and widely applied antibiotics in veterinary medicine. These compounds form metal(II) complexes of various compositions and structures depending on the antibiotic form used in the synthetic procedure (acidic or sodium) and on the nature of the metal(II) ion [2-9]. To the best of our knowledge there are no literature data regarding any toxicology information about metal(II) complexes of polyether ionophores. The following compounds were used in the present study:

- sodium monensin (MonNa), $[Mn(MonNa)_2Cl_2]$, $[Co(MonNa)_2Cl_2]$,
- monensic acid (MonH), $[Co(Mon)_2(H_2O)_2]$, $[Zn(Mon)_2(H_2O)_2]$,
- sodium salinomycin (SalNa), $[Mg(Sal)_2(H_2O)_2]$, $[Ca(Sal)_2(H_2O)_2]$, $[Co(Sal)_2(H_2O)_2]$ and $[Zn(Sal)_2(H_2O)_2]$.

The complexes of sodium monensin with Mn(II) and Co(II) have distorted tetrahedral geometry, while the rest of the divalent metal compounds are isostructural with an octahedral environment of the metal(II) ion.

Table 1. LD_{50} of monensin, salinomycin and some of their metal complexes (ICR mice, *per os*, 24 h treatment)

Compound	LD_{50} (confidence interval)	
	mg / kg b.w.	μmol / kg b.w.
MonNa *	> 100	> 144
$[Mn(MonNa)_2Cl_2]$ *	> 79.4	> 52
$[Co(MonNa)_2Cl_2]$ *	> 31.6	> 21
MonH	87.0 (63-120)	130 (94-179)
$[Co(Mon)_2(H_2O)_2]$ *	> 31.6	> 22
$[Zn(Mon)_2(H_2O)_2]$	34.2 (23-51)	24 (16-35)
SalNa	21.6 (15-32)	28 (19-41)
$[Mg(Sal)_2(H_2O)_2]$	25.8 (21-32)	17 (13-21)
$[Ca(Sal)_2(H_2O)_2]$	20.5 (17-25)	13 (11-16)
$[Co(Sal)_2(H_2O)_2]$	44.7 (38-60)	27 (23-37)
$[Zn(Sal)_2(H_2O)_2]$	108.0 (73-160)	67 (46-100)

" * " - to be precisely determined

Table 2. Representative biochemical indices of survivals (ICR mice, *per os*, 72 h treatment)

Compound	Dose, mg/kg b.w.	ALB, g/L	TP, g/L	AST, U/L	ALT, U/L	ALP, U/L	LDH, U/L	CR, μmol/L	URE, mmol/L
$[Mg(Sal)_2(H_2O)_2]$	20.0	36.6	-	640	91	362	1435	43.4	-
	25.0	29.4	-	173	68	203	1888	-	-
	31.6	33.0	-	385	83	330	905	62.2	-
	50.1	28.1	-	510	93	391	1750	-	-
$[Ca(Sal)_2(H_2O)_2]$	10.0	35.8	-	178	58	228	796	-	-
	12.6	34.3	60.0	240	95	216	-	66.5	12.7
	15.8	34.8	64.5	173	73	316	-	60.5	11.5
	20.0	43.7	-	175	95	219	954	37.2	-
	25.0	40.3	55.6	280	108	317	1571	40.4	10.2
	31.6	37.9	51.1	705	305	412	-	44.1	-
	39.8	34.6	63.4	255	70	129	-	55.3	13.9
	50.1	50.3	-	385	170	324	1255	43.1	-
Controls		23-34	50-74	95-474	32-105	150-326	695-2634	27-59	5-17

" - " – not determined

The data on the acute toxicity of the compounds tested are presented in Table 1. As it can be seen, the most toxic compounds are $[Ca(Sal)_2(H_2O)_2]$ and $[Mg(Sal)_2(H_2O)_2]$, whereby MonH is the least toxic among all substances studied with precisely determined LD_{50} values. It should be mentioned that Zn(II) analogues of monensin and salinomycin differ significantly in their toxicity, as compared to the starting antibiotics. Thus monensic acid is five times less toxic than $[Zn(Mon)_2(H_2O)_2]$, while sodium salinomycin possesses two-fold increased acute toxicity if juxtaposed with $[Zn(Sal)_2(H_2O)_2]$.

The clinical signs of the treated animals depend both on the type and concentration of compounds studied. The death of animals treated with MonH and MonNa is not accompanied by any behavior changes and other symptoms, while the treatment with $[Zn(Mon)_2(H_2O)_2]$ leads to adynamia, bradypnea, loss of postural reflex, clonic seizures. The treatment with low doses of SalNa during the first 2-3 hours is accompanied by increased physical activity, while at high doses decreased physical activity and tachypnea were observed. Later ataxia, loss of postural reflex and aggressive behavior in survived animal groups were noticed. The compounds $[Co(Sal)_2(H_2O)_2]$ and $[Zn(Sal)_2(H_2O)_2]$ do not provoke significant changes in the animal status at the beginning – the animals are agitated with increased physical activity. After several hours, considerable alterations are noted – mice become oppressed, drowsy and lose postural reflex. In the group treated with $[Mg(Sal)_2(H_2O)_2]$ and $[Ca(Sal)_2(H_2O)_2]$ tremor, disorientation and ataxia are observed. All clinical signs of toxicity are indicative for central nerve system (CNS) toxic effect of high doses of antibiotics administered (acute toxicity).

Salinomycin complexes with ions of Mg(II) and Ca(II) showed highest toxicity, and for that reason we studied their effect on animals by additional biochemical assays using survivals. For this purpose a three-day treatment was applied with the same dose of the given compound used during acute toxicity experiments. Representative data on the parameters tested (ALB, TP, AST, ALT, ALP, LDH, CR, URE) upon treatment with salinomycin complexes are summarized in Table 2.

From the clinical chemistry results obtained it can be concluded that 72 h treatment (once per day) with salinomycin metal(II) complexes does not significantly influence most of the paraclinic laboratory parameters of the treated animals and the differences with the control group are not significant. The normal serum creatinine and urea values are indicative that there is no acute kidneys damage displaying renal insufficiency. Increased ALB in the group treated with $[Ca(Sal)_2(H_2O)_2]$ can result from dehydration of the animals (referred to behavior changes of intoxicated animal) followed by subsequent hemoconcentration. The increased AST values measured for some animals suggest possible myocardial damage when salinomycin complexes were applied because the toxicity mechanism of the tested compounds is related to ion-channel disturbances in the myocardium. However, more detailed studies on the myocardium toxicity related to high doses of these compounds must be performed combined with histological

studies. The liver function seems to be intact for the period of testing without serious disturbances.

From the biochemical analyses performed it can be concluded that a three-day treatment with Mg(II) and Ca(II) complexes of salinomycin does not affect liver and kidneys functions. Possible myocardium dysfunction is suggested, but to have more deep insights into the mechanism of toxicity resulting from application of antibiotics complexes, a prolonged treatment (chronic/sub-chronic toxicity studies) should be performed.

CONCLUSION

The LD_{50} values of monensin, salinomycin and their metal complexes were determined on ICR mice. The data showed that the least toxic compound among the substances studied is monensic acid, whereby the Ca(II) and Mg(II) complexes of salinomycin are the most toxic ones. The 72 h treatment of animals does not significantly change most of the biochemical parameters. From the clinical signs of toxicity observed and based on the preliminary biochemical data obtained it can be suggested that the lethal outcome is associated with CNS toxicity and breath insufficiency.

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IN VIVO ТОКСИЧНОСТ НА МОНЕНЗИН, САЛИНОМИЦИН И ТЕХНИ МЕТАЛНИ КОМПЛЕКСИ (ПРЕДВАРИТЕЛНО ИЗСЛЕДВАНЕ)

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Изследвана е острата токсичност на полиетерните йонофори монензин, салиномицин и комплексите им с Na(I), Mg(II), Ca(II), Mn(II), Co(II), Zn(II) върху мишки, порода ICR. Установено е, че от тестваните съединения Ca(II) и Mg(II) комплекси на салиномицин са най-токсични със стойности за LD_{50} 20.5 mg/kg b.w. (13 μmol/kg b.w.) и 25.8 mg/kg b.w. (17 μmol/kg b.w.), съответно. Предварителната оценка показа, че тридневното третиране с полиетерни йонофорни антибиотици и техните комплекси не води до съществени промени в биохимичните показатели на преживелите животни.