

EFFECTS OF REPEATED ADMINISTRATION OF DITHIOL CHELATING AGENT - SODIUM 2,3-DIMERCAPTO-1-PROPANESULPHONATE (DMPS) - ON BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN RABBITS

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Summary: The effects of weekly intravenously administered a dithiol chelating agent - sodium 2,3-dimercaptopropane-sulphonate (DMPS) - in a single dose of 50 mg/kg/week for 10 weeks on biochemical and haematological parameters were studied in rabbits. DMPS was well tolerated, an increase in body weight was similar in the DMPS-treated and control animals. DMPS caused significant decrease in plasma calcium and vitamin E concentrations at the end of the experiment. No significant differences in haematological parameters between the DMPS and control groups were observed. A significant decrease in magnesium content in myocardial tissue was observed in the DMPS-treated rabbits. The above-mentioned biochemical changes should be taken into account in studies of possible chelating and radical scavenging effects of DMPS in various pathological conditions.

Key words: 2,3-dimercapto-1-propanesulphonate; DMPS; Chelating agents; Dithiols; Biochemistry; Haematology; Myocardial elements; Rabbit

Introduction

Early in the eighties it was shown that some newer complexing agents, e.g. sodium 2,3-dimercapto-1-propanesulphonate (DMPS; unithiol), or meso-2,3-dimercaptosuccinic acid (DMSA), were effective in mercury, arsenic and lead poisoning. Compared to 2,3-dimercaptopropanol (BAL), the newer agents were of significantly lower toxicity. Moreover, they can be administered orally or intravenously (1). Thus, it can be expected that older chelating agents (e.g. BAL, CaNa₂EDTA) will be replaced by DMPS and DMSA (2). Effectiveness of the first mentioned agent - DMPS - was also demonstrated in various experimental heavy metal intoxications, e.g. in As, Hg, Au intoxications (8,23,15).

In addition to heavy metal-chelating activity, DMPS as a dithiol agent may act as an oxygen radical scavenger and

thus it may inhibit lipid peroxidation (4,3). Oxidative stress plays an important role in various pathological conditions, e.g. in the anthracycline cardiotoxicity (20). In relation to the study of DMPS in the above-mentioned pathological state, we have studied changes of biochemical and haematological parameters during the course of repeated (10 weeks) intravenous DMPS administration in rabbits. In addition, myocardial content of some elements (calcium, potassium, magnesium, iron, selenium) was also measured at the termination of the experiment.

Methods

Chemicals

2,3-dimercaptopropane-1-sulfonic acid, sodium salt (Sigma Chemie, Czech Republic); ketamin (Narkamon 5% inj., Léčiv-

va, Czech Republic); pentobarbital (Nembutal Sodium, Abbott, USA); aqua pro injectione (Biotika, Slovakia), saline (Solutio natrii chlorati isotonica, Biotika, Slovakia).

Experimental design

Medium size Chinchilla male rabbits of average weight 3.0 kg and age 4 months at the beginning of the experiment were used. This experiment followed the Law of the Czech National Council for the protection of animals against cruelty, as well as the European convention for the protection of vertebrate animals used for experimental and other scientific purposes of the Council of Europe, and was under the supervision of the Ethics Committee of the Medical Faculty, Charles University, Hradec Králové.

DMPS was administered i.v. to twelve rabbits in a dose of 50 mg/kg once weekly for 10 weeks. DMPS was dissolved in aqua pro injectione immediately before administration in a concentration 50 mg/ml. Fifteen control rabbits were given saline i.v. in corresponding volume (1.0 ml/kg). The weight of rabbits was monitored during the experiment. Biochemical and haematological parameters were determined in arterial blood samples (plasma, or serum, resp.) before the 1st and 5th administration of the drug and at the end of the experiment (3-5 days after the 10th administration of the drug). After the sacrifice of rabbits by i.v. pentobarbital overdosing at the 11th week, the gross autopsy was performed, heart was excised, sample of the left ventricle was removed for determination of elements content - calcium, potassium, magnesium, iron and selenium.

Biochemical parameters

The following biochemical parameters were determined in plasma (serum) samples using automatic analyser Hitachi 717 (Japan): Na, K, Ca, Cl, Mg, phosphate, glucose, urea, creatinine, uric acid, bilirubin, lactate dehydrogenase (LD), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), alkaline phosphatase (ALP), cholesterol, triglycerides, proteins incl. electrophoresis, glutathione peroxidase (GSH-px), glutathione (GSH), malone dialdehyde (MDA), vitamin E.

Haematological parameters

Parallel to biochemical parameters, the following haematological parameters were determined using analyser Coulter T890 (USA): white blood cells count and white blood picture, red blood cells count, haemoglobin, haematocrit and thrombocytes count.

Myocardial content of elements

The content of calcium (Ca), potassium (K), magnesium (Mg), iron (Fe) and selenium (Se) was measured in samples of dry, mineralized (using microwave digestion with nitric acid and hydrogen peroxide in microwave oven, Milestone, Italy) left ventricular tissue and expressed in $\mu\text{mol/g}$ (Se in nmol/g) of dry tissue. Ca, Mg and Se were determined by atomic absorption spectrophotometry using

an analyser Unicam Sollar 959 (USA). Content of K and Fe was measured photometrically using an apparatus Eppendorf Efox 5053 (Germany), and Hitachi 717 (Japan), resp.

Statistical analysis

The data are presented as means \pm S.E.M. Significance was estimated with the adequate t-test at the level $p \leq 0.05$ (9).

Results

Body weight

The initial values of body weight were 3193 ± 68 g in the control group, and 3042 ± 60 g in the DMPS group, resp. The weight gain in both groups of rabbits was almost identical during the experiment (Fig. 1).

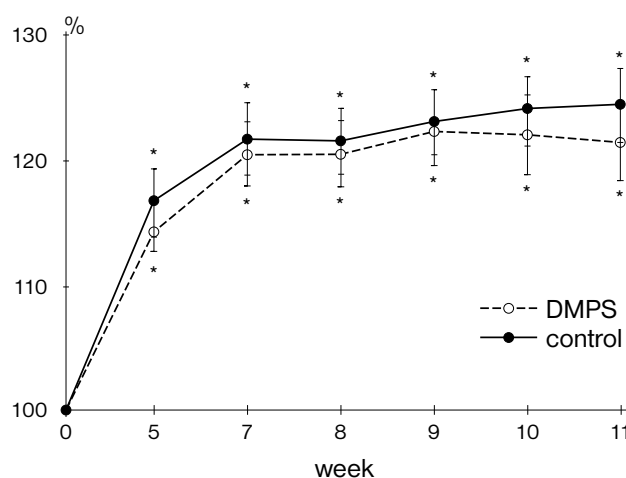


Fig. 1: Body weight gain (%) in the control and DMPS treated rabbits during the experiment (11 weeks); $p < 0.005$ compared to the initial value.

Biochemical parameters

As shown in tab. 1, there were some significant differences between control and DMPS treated animals at the beginning of the experiment in some measured biochemical parameters. In the DMPS group, there was a higher concentration (or activity) of Na, urea, ALT, albumin, GSH-px and GSH, compared to the control group. No consistent trends were found in the measured parameters during the experiment (i.e., in the 5th week). At the end of the experiment, a significant decrease in Ca plasma concentration was found in DMPS treated rabbits compared to the control group. In addition, there was a significantly lower increase in CK activity in DMPS group vs. control group. In the middle of the experiment (the 5th week), there was also transient, but significant increase in some globulin fractions (α_2 , β , γ) of plasma proteins in DMPS treated rabbits. Compared to the initial values, there was a significant decrease in vitamin E concentration at the end of the experiment in the DMPS group.

Table 1: Biochemical parameters (initial = absolute values) and their changes (%) during repeated i.v. administration of DMPS (50 mg/kg/week).

Parameter	Time interval (weeks) control group (n ₁ =15) DMPS group (n ₂ =12)		
	1 (initial)	5	11 (final)
sodium (mmol/l)	141.7±0.7 144.5±0.7 [#]	101.2±0.7 99.0±0.7 [#]	101.9±0.8* 99.8±1.2
potassium (mmol/l)	4.0±0.2 3.9±0.1	93.6±3.2 92.4±4.1	83.9±5.0* 106.0±11.6
chloride (mmol/l)	102.2±1.0 104.3±0.8	102.4±1.2 97.7±1.0 ^{#*}	100.6±1.5 103.2±2.7
calcium (mmol/l)	3.1±0.1 3.2±0.1	102.7±4.6 106.4±2.2*	96.5±2.2 86.8±4.1 ^{#*}
magnesium (mmol/l)	0.94±0.08 1.02±0.03	359.3±253.0 96.5±8.7	893.6±792.8 99.9±4.0
phosphate (mmol/l)	1.90±0.10 1.85±0.07	83.1±3.9* 85.8±3.8*	77.2±4.7* 75.2±2.9*
glucose (mmol/l)	10.8±0.8 8.7±0.9	100.3±9.2 118.8±13.3	133.7±13.8* 131.0±14.4
urea (mmol/l)	7.9±0.3 9.7±0.3 [#]	111.7±6.3 95.4±4.4	101.4±4.8 92.7±8.0
creatinine (μmol/l)	91.2±4.1 90.9±1.9	106.9±2.8* 106.9±3.8	108.4±4.8 109.9±5.3
uric acid (μmol/l)	12.5±1.8 13.9±2.3	72.3±11.6* 83.2±34.5	100.8±34.4 94.9±53.3
bilirubin (μmol/l)	6.3±0.3 6.3±0.2	86.1±3.5* 81.0±2.4*	84.0±3.4* 80.2±2.9*
LD (μkat/l)	7.1±2.0 7.8±1.0	249.1±80.3 121.8±26.5	300.3±139.0 108.8±19.7
ALT (μkat/l)	1.2±0.1 1.8±0.1 [#]	102.1±6.6 83.9±3.9 ^{#*}	92.2±7.4 75.1±4.3*
AST (μkat/l)	0.49±0.05 0.58±0.04	146.7±16.8* 104.8±8.3 [#]	114.0±9.5 118.2±15.7
CK (μkat/l)	23.6±4.0 32.4±3.8	134.8±22.0 105.0±14.1	202.4±24.9* 124.2±18.1 [#]
ALP (μkat/l)	3.4±0.3 2.9±0.2	102.1±11.4 85.9±6.1*	53.9±6.9* 55.7±5.3*
cholesterol (mmol/l)	1.04±0.11 1.15±0.09	119.9±11.4 94.2±5.6	79.4±8.3* 214.6±145.4
triglycerides (mmol/l)	1.03±0.09 0.85±0.09	91.7±8.0 137.8±18.4 [#]	133.1±13.0* 168.7±19.4*

Table 1 (cont'd)

protein (g/l)	62.3±1.1 64.7±0.8	107.4±2.7* 97.7±5.8	99.7±2.5 101.3±3.4
electrophoresis:			
albumin (%)	66.3±1.3 69.8±1.3 [#]	103.1±1.1* 95.1±2.0 [#]	101.2±2.6 98.1±2.0
α ₁ globulin	5.6±0.6 6.2±0.2	140.9±50.8 see note	117.6±22.5 96.3±8.6
α ₂ globulin	6.7±0.4 5.2±0.4 [#]	92.3±5.3 138.4±11.1 ^{#*}	97.6±7.0 122.5±9.2 ^{#*}
β globulin	11.0±1.1 9.3±1.0	109.0±11.4 184.3±14.9 ^{#*}	87.7±8.2 95.6±8.7
γ globulin	12.5±0.9 10.6±0.5	88.9±7.1 110.6±6.4 [#]	102.7±8.8 111.0±7.5
A/G quotient	2.04±0.13 2.51±0.14 [#]	114.9±5.9 see note	103.5±11.4 90.6±6.1
GSH-px (U/g Hb)	84.0±7.8 124.2±9.9 [#]	216.9±22.7* 223.8±13.2*	378.2±40.4* 376.4±26.7*
GSH (mg/g Hb)	5.4±1.2 9.6±1.2 [#]	511.1±222.3 181.4±123.0	414.2±176.1 240.6±139.1
MDA (μmol/l)	0.86±0.11 0.87±0.10	92.6±12.3 99.2±10.2	116.7±22.1 102.0±10.0
vitamin E (μmol/l)	4.3±1.0 2.6±0.4	74.4±11.5 60.0±20.6	97.6±25.0 58.9±15.2*

LD - lactate dehydrogenase;
 ALT - alanine aminotransferase;
 AST - aspartate aminotransferase;
 CK - creatine kinase;
 ALP - alkaline phosphatase;
 A/G quotient - albumin/globulin quotient;
 GSH-px - glutathione peroxidase;
 GSH - glutathione;
 MDA - malondialdehyde.

Note: values not measured due to interference between fractions of albumin and α₁ globulin.

Statistical significance (p ≤ 0.05):

* - compared to the initial value;

- between groups

Haematological parameters

Tab. 2 shows that there were no significant differences in haematological parameters between the DMPS and control groups of rabbits at the beginning of the experiment. Some changes observed during the experiment were comparable in both groups.

Table 2: Haematological parameters (initial = absolute values) and their changes (%) during repeated i.v. administration of DMPS (50 mg/kg/week).

Parameter	Time interval (week) control group (n ₁ =15) DMPS group (n ₂ =12)		
	1 (initial)	5	11 (final)
leucocytes (10 ⁹ /l)	7.9±0.7 7.6±0.6	90.1±7.2 89.9±6.9	57.4±5.9* 61.1±6.4*
erythrocytes (10 ¹² /l)	5.8±0.2 5.9±0.1	109.4±2.1* 110.3±2.7*	108.8±2.9* 111.7±1.5*
haemoglobin (g/l)	120.9±3.0 121.3±2.4	113.2±1.9* 115.1±3.3*	110.5±2.8* 117.4±2.2*
haematocrit (ratio)	0.377±0.081 0.394±0.008	112.1±2.3* 111.7±2.3*	111.1±3.0* 112.6±2.1*
MCV (fl)	65.7±0.5 66.4±0.5	102.6±1.1* 101.2±1.1	102.0±0.7* 100.6±0.9
thrombocytes (10 ⁹ /l)	544.3±34.6 595.3±42.2	84.7±4.9* 75.9±5.3*	83.7±3.9* 78.6±6.3*
<u>differential count</u> (%)			
band neutrophils	0.5±0.2 0.1±0.1	0.0±0.0 0.0±0.0	0.0±0.0 12.5±12.5*
segment neutrophils	41.5±3.5 40.3±4.8	97.4±12.1 98.6±16.6	104.5±18.2 125.1±19.5
eosinophils	2.5±1.0 1.2±0.5	19.7±12.1* 287.5±138.1	140.7±45.2 25.0±17.1#*
basophils	1.7±0.8 0.6±0.2	75.0±46.1 10.0±10.0*	72.2±25.7 66.7±66.7
monocytes	2.7±0.7 2.0±0.5	88.8±14.2 88.3±37.5	203.6±56.4 98.3±46.3
lymphocytes	48.5±3.8 55.8±4.9	134.4±11.8* 117.6±13.5	111.2±13.0 88.2±10.9

MCV- mean cellular volume.

Statistical significance (p ≤ 0.05):

* - compared to the initial value;

- between groups

Myocardial content of elements

There was a tendency to a decrease in calcium and potassium content in the myocardium of DMPS treated rabbits. In the case of magnesium, this decrease was significant. The content of iron and selenium were not significantly different in both groups of rabbits (tab. 3).

Table 3: The content of elements - calcium, potassium, magnesium, iron and selenium - in the left cardiac ventricle after repeated i.v. administration of DMPS (50 mg/kg/week).

Group	Ca	K	Mg	Fe	Se
control (n ₁ =15)	17.7±3.1	436.9±69.7	65.1±6.8	9.6±1.8	10.0±1.2
DMPS (n ₂ =12)	11.2±1.3	390.5±29.5	47.6±4.6#	9.5±1.4	12.7±1.5

Means ± S.E.M. in μmol/g (Se-nmol/g) of dry tissue.

Statistical significance between groups: # p ≤ 0.05

Discussion

Repeated i.v. administration of DMPS in a cumulative dose of 0.5 g/kg/10 weeks was well tolerated by rabbits. The weight gain of the DMPS treated animals was comparable with that of the control group. After slow i.v. administration of a single dose 50 mg/kg of DMPS only transient irregularities in respiration were observed. These results correspond to those of other authors who had studied chronic effects of DMPS in other species of experimental animals (19, 22). Rabbits were used in our experiment considering a follow-up study of possible effect of DMPS on anthracycline cardiotoxicity, as the anthracycline model of chronic heart failure in rabbits has been often used for these purposes (24,5,10). Similarly, the selected single dose of DMPS (i.e. 50 mg/kg) corresponds on a molar basis to the doses of both experimentally and clinically used cardioprotective agent against anthracycline cardiomyopathy - dexrazoxane (ICRF-187) (12).

In some measured biochemical parameters (Na, urea, ALT, GSH-px, etc.) there were significant differences between the DMPS and control groups at the beginning of the experiment. This fact can be explained by the variation of the biochemical parameters in rabbits (17). A significant decrease in plasma calcium concentration in the DMPS treated rabbits may be possibly due to chelating activity of DMPS. The concentration of other ions, however, was not affected by DMPS treatment. An increase in CK activity in both groups of rabbits during the experiment can be explained by repeated i.m. administration of an anaesthetic agent (ketamine). In the DMPS group this increase was significantly attenuated at the end of the experiment.

The cause of a significant decrease in vitamin E plasma concentration at the end of the experiment in the DMPS treated rabbits (compared to the initial value) may be complex. Rapid oxidation of DMPS after i.v. administration to disulfide forms (16,18) in the blood is supported by kinetic data. Fifteen minutes after i.v. administration of DMPS (3.0 mg/kg) to humans only 12% of the total DMPS was unchanged, i.e. nearly 90% of DMPS was oxidized to disulfides (14). In the cells disulfide forms of DMPS can be

reduced at the expense of vitamin E or by involving glutathione-disulfide exchange reaction (21). In addition, depletion of cytosol GSH may contribute to the attenuated regeneration of the membrane-bound vitamin E (7,13).

Doses of DMPS used in this study caused no significant changes in haematological parameters. Higher doses of DMPS can cause anaemia due to copper deprivation, as observed by Szinich et al. (22) in dogs treated i.v. with DMPS 2 x 75 mg/kg daily for 10 weeks.

In the DMPS group, there was a significant lower content of Mg in the myocardium of the left ventricle. There was also tendency to a decrease in Ca and K myocardial content, in the case of Ca the decrease was near the level of statistical significance ($p = 0.069$). On the other hand, the content of Fe and Se was not practically affected by DMPS treatment. The described changes in mineral concentrations, especially those concerned magnesium, calcium and partially iron, were consistent with findings of Bosque, et al. (1990).

In conclusion, experimental data of our study confirmed low toxicity of repeatedly i.v. administered DMPS in rabbits. In respect to a possible use of this dithiol chelating agent in other pathological states than heavy metal intoxications (e.g. in those where an oxidative stress plays a role), one should take into account distinct effects of DMPS on the myocardial content of some important elements, particularly of magnesium and calcium.

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