ORIGINAL PAPERS

Adv Clin Exp Med 2006, **15**, 5, 767–776
ISSN 1230-025X

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Morin-5'-Sulfonic Acid Sodium Salt as an Antidote in the Treatment of Acute Chromium Poisoning in Rats

Sól sodowa kwasu moryno-5'-sulfonowego jako odtrutka w leczeniu ostrego zatrucia związkiem chromu u szczurów

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Abstract

Background. Cr(VI) ions exhibit greater toxicity than Cr(III) ions since they easily cross biological membranes and have caustic properties. *In vitro* studies demonstrated the ability of morin-5'-sulfonic acid sodium salt (NaMSA) to reduce Cr(VI) ions to the less toxic Cr(III) ions. Therefore it can be expected that administering NaMSA in chromium(VI) poisoning will promote the reduction of chromium ions from the oxidation state of +6 to +3, which would alleviate the course of the intoxication.
Objectives. Evaluating the efficacy of NaMSA in the treatment of acute Cr(VI) poisoning.
Material and Methods. Experiments were performed on Wistar rats, which were divided into a control group (K) and four test groups (A, B, C, D). All the animals were administered chromium trioxide (CrO_3) intragastrically at its LD_{50} level. The antidote (NaMSA) was administered intragastrically once at 50 mg/kg (group A) or 100 mg/kg (group B) 30 min after treatment with the poison. In the remaining groups, NaMSA was injected intraperitoneally two hours after poison administration, and then twice a day for four days at 50 mg/kg (group C) or 100 mg/kg (group D).
Results. The activities of aminotransferases and the concentrations of bilirubin, urea, and creatinine in blood were observed to increase in groups K, C, and D, which indicates damage to the parenchyma of organs. There was no reduction in mortality in groups A, C, and D in comparison with the control group. A reduction in mortality was observed only in group B.
Conclusion. Treatment of acute CrO_3 poisoning in rats was effective in decreasing the mortality of poisoned animals, lowering damage to the parenchyma of organs, and suppression of chromium absorption only when NaMSA was administered intragastrically at a dose of 100 mg/kg. NaMSA's mechanism of detoxification is most probably based on the reduction of Cr(VI) ions to Cr(III) ions, which do not show caustic properties and are poorly absorbed from the digestive tract (**Adv Clin Exp Med 2006, 15, 5, 767–776**).

Key words: chromium trioxide, acute poisoning, NaMSA, rats.

Streszczenie

Wprowadzenie. Sześciowartościowe jony chromu Cr(VI) wykazują większą toksyczność niż jony Cr(III), ponieważ łatwiej przenikają przez błony biologiczne i mają właściwości żrące. Badania *in vitro* wykazały, że sól sodowa kwasu moryno-5'-sulfonowego (NaMSA) redukuje jony Cr(VI) do mniej toksycznych jonów Cr(III). Dlatego można przypuszczać, że podanie NaMSA w zatruciu chromem sześciowartościowym spowoduje redukcję jonów Cr(VI) do jonów Cr(III), co mogłoby wpłynąć korzystnie na przebieg zatrucia.
Cel pracy. Określenie skuteczności NaMSA jako odtrutki w ostrym zatruciu chromem sześciowartościowym.
Materiał i metody. Badania przeprowadzono na szczurach szczepu Wistar podzielonych na grupę kontrolną K i 4 grupy badane (A, B, C, D). Wszystkie zwierzęta otrzymały dożołądkowo (*i.g.*) dawkę LD_{50} trójtlenku chromu (CrO_3). Po 30 min podano *i.g.* odtrutkę (NaMSA) w dawce 50 mg/kg (grupa A) lub 100 mg/kg (grupa B).

W pozostałych grupach NaMSA wstrzyknięto dootrzewnowo 2 godz. po podaniu trucizny, a następnie kontynuowano podawanie przez 4 dni 2 razy dziennie w dawce 50 mg/kg (grupa C) lub 100 mg/kg (grupa D).
Wyniki. W grupach K, C i D wzrosła aktywność aminotransferaz, stężenie bilirubiny, mocznika i kreatyniny, co wskazuje na uszkodzenie narządów miąższowych. Nie stwierdzono zmniejszenia śmiertelności w grupach A, C i D w porównaniu do grupy K. Śmiertelność zatrutych zwierząt obniżyła się jedynie w grupie B.
Wnioski. Leczenie ostrego zatrucia CrO_3 spowodowało obniżenie śmiertelności, zmniejszenie uszkodzenia narządów wewnętrznych oraz wchłaniania chromu z przewodu pokarmowego jedynie w grupie szczurów otrzymujących NaMSA w dawce 100 mg/kg (grupa B). Działanie NaMSA jako odtrutki wynika najprawdopodobniej z redukowania jonów Cr(VI) do jonów Cr(III), które nie wykazują działania żrącego i gorzej wchłaniają się z przewodu pokarmowego (**Adv Clin Exp Med 2006, 15, 5, 767–776**).

Słowa kluczowe: trójtlenek chromu, ostre zatrucie, NaMSA, szczury.

Acute chromium poisoning is characterized by a dramatic clinical course and high mortality. Hexavalent chromium compounds show much greater toxicity than its trivalent compounds since chromium ions in the +6 oxidation state of easily cross biological membranes and exhibit caustic and oxidative properties [1]. When chromium ions are ingested, they very quickly provoke intense hemorrhagic gastroenteritis, leading to circulatory failure, while in persons who survive the initial stage of poisoning they accumulate in the parenchyma of organs, often causing dangerous liver and kidney damage or even insufficiency of these organs [1–3].

Treatment of acute chromium poisoning is very difficult since we have no efficient antidote. Compounds binding chromium that has been absorbed, so-called chelating compounds (e.g. calcium disodium versenate), are not very effective and very toxic. Methods of extracorporeal elimination of chromium ions from serum, such as hemodialysis and hemoperfusion, are also not efficient enough [1]. Some believe that both chelating compounds and the methods of extracorporeal elimination of the poison are completely ineffective in acute chromium poisoning [3]. It has recently been postulated that a compound capable of reducing chromium from the oxidation state of +6 to the less toxic oxidation state of +3 would be more promising in the treatment of acute chromium poisoning. Such substances as ascorbic acid, glutathione, cysteine, cysteamine, lipoic acid, and coenzymes A and M are able to reduce chromium ions strongly under *in vitro* but not *in vivo* conditions [4]. *In vitro* studies have also indicated that some substances belonging to the polyhydroxyflavones, e.g. morin (3,5,7,2',4'-pentahydroksyflavone) and quercetin (3,5,7,3',4'-pentahydroksyflavone) and their sulfonic derivatives, characterized by good aqueous solubility, such as quercetin-5'-sulfonic acid sodium salt (NaQSA) and morin-5'-sulfonic acid sodium salt (NaMSA), can reduce chromium ions from the oxidation state of +6 to +3. These substances not only reduce metal cations from higher to lower oxidation states, but also form complexes with metal ions [5, 6]. Experimental studies showed that NaQSA could be an efficient antidote in acute chromium poisoning [7] as well as in chronic mercury, cadmium, and lead poisoning [8]. Therefore it can be expected that administration of NaMSA in chromium(VI) poisoning will promote the reduction of chromium ions from the oxidation state of +6 to +3, which would alleviate the course of the intoxication.

The aim of this study was to evaluate the effectiveness of NaMSA in the treatment of experimental acute poisoning with a hexavalent chromium compound, chromium trioxide (CrO_3), in rats. The effect of NaMSA treatment on mortality and biochemical indicators of hepato- and nephrotoxicity in acute CrO_3 poisoning were assessed. In addition, chromium concentrations in the blood and internal organs (liver and kidney) were analyzed.

Material and Methods

Morin-5'-sulfonic Acid Sodium Salt: Properties, Synthesis, and Determination of Acute Toxicity

Morin (3,5,7,2',4'-pentahydroxyflavone) is a natural dye of plant origin. Its chemical structure determines its biological activity in plants, animals, and humans, including its bactericidal and antioxidant properties and protection from UV radiation [5]. Morin has been applied in chemical analysis for spectrophotometric and fluorometric assays of metals [9]. However, the usefulness of morin is limited by its virtual insolubility in water. A sulfonic derivative of morin, NaMSA, is easily soluble in water and preserves the activities of the parent compound. The sulfonic group in the morin molecule does not significantly change the acidic properties of the OH groups, but increases the acidity of the whole compound. The solubility of NaMSA in water at 22°C ± 1°C (295 K) is $2.7 \cdot 10^{-2}$ mol/dm^3, while the density of its saturated solution

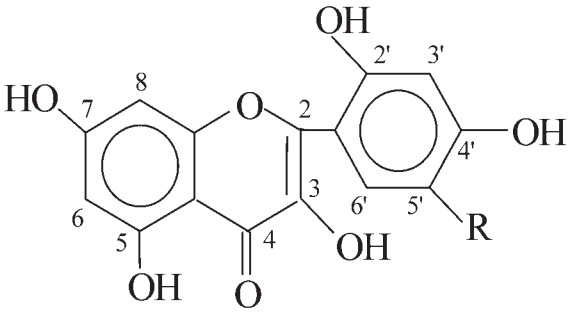

Fig. 1. Formulae of morin (R = H) and morin-5'-sulfonic acid sodium salt (R = SO_3Na)

Ryc. 1. Wzór moryny (R = H) i soli sodowej kwasu moryno-5'-sulfonowego (R = SO_3Na)

under the same conditions is 1.003 g/cm^3. NaMSA is a multiprotonic acid which in aqueous solution gradually undergoes acidic dissociation (K_a) with rising pH, whereas in a strongly acidic solution it is protonated. The NaMSA pK_a values determined potentiometrically in aqueous solutions at 20°C and I = 0.1 are: pK_{a1} = 4.67, pK_{a2} = 7.84, pK_{a3} = 9.82, and pK_{a4} = 10.69 [5, 10].

The NaMSA used for the toxicity studies was obtained according to the method described earlier [9]. The purity of the product was checked with thin-layer chromatography on alumina plates covered with an adsorbent (silica gel 60 WF_{254s}, MERCK). The mobile phase was composed of n-butanol:acetic acid:water (4:1:5). It was demonstrated that NaMSA obtained by synthesis was a homogenous substance and did not contain untransformed morin. The molecular formula of the product was determined by elemental analysis for C, H, and S contents; the number of crystalline water molecules was established by the gravimetric and derivatographic method, while sodium content was measured with atomic absorption spectrometry. The electronic absorption spectrum of aqueous NaMSA solution between 200–800 nm showed two bands from π–π^* electronic transitions. Band I was seen at λ_I = 370 nm (ε = 12,700) and band II at λ_{II} = 262 nm (ε = 20,500). The spectrophotometric characteristics of this NaMSA preparation are in agreement with literature data [9]. The obtained result confirm the identity of the product of morin sulfonation as morin-5'-sulfonic acid sodium salt with the molecular formula $C_{15}H_9O_{10}SNa{\cdot}2H_2O$ and a molar mass of 440.319.

Before evaluating NaMSA's efficacy as an antidote in chromium poisoning, we determined its toxicity. NaMSA and NaQSA have a very similar chemical structure, so it could be expected that their toxicity is comparable. The toxicity of NaQSA was calculated earlier using the Lichfield and Wilcoxon method; LD_{50} of this compound in rats exceeds 2000 mg/kg [8]. The study of the toxicity of NaMSA was conducted with the Acute Toxic Class Method since, though this method requires a small number of animals, it allows for the classification of a xenobiotic according to commonly accepted systems [11].

Toxicity was first tested on 9 males (3 animals per dose) by intragastric administration of a NaMSA (25, 200, 2000 mg/kg b.w.) suspension in normal saline solution through a metal tube. Then the experiment was repeated by administering the same doses to 9 females [11]. The conditions under which the animals were kept were the same as in the experiments to evaluate the efficacy of NaMSA in the treatment of acute chromium poisoning. No animal deaths were observed. After 14 days of observation, the animals were examined at autopsy and their livers and kidneys were examined under a microscope. Microscopic examination did not reveal any abnormalities. Therefore it was confirmed that NaMSA was practically nontoxic to rats, and its LD_{50} was above 2000 mg/kg b.w., as is NaQSA's.

Evaluation of the Efficacy of Morin-5'-sulfonic Acid Sodium Salt in the Treatment of Experimental Acute Chromium Poisoning in Rats

Since NaQSA and NaMSA have similar molecular structures and toxicity, the experiment was conducted in a model described previously for studies of NaQSA efficiency as an antidote in CrO_3 poisoning. The NaMSA doses were also the same as the NaQSA doses used earlier [7]. Acute poisoning with a hexavalent chromium compound was induced by administering CrO_3 (Sigma, cat. no. C-6051) to the animals. CrO_3 was chosen because of its excellent solubility in water (up to 62 g/100 ml) [12] and already established LD_{50} in rats (80 mg/kg b.w.) [7, 12].

The study was conducted on 100 Wistar rats of both sexes with an average body weight of 200 ± 12.8 g. The animals were kept under standard conditions with water and pellet food (LSM, "Agropol" Motycz) available *ad libitum*. The rats were divided into a control group (K) and four test groups (A, B, C, and D). Each group comprised 20 animals, half of which were females and the other half males. The animals were observed for 14 days after poison administration [11].

Group K animals were administered CrO_3 at LD_{50} once intragastrically. Group A animals were administered CrO_3 at LD_{50} once intragastrically.

NaMSA at a dose of 50 mg/kg b.w. was administered intragastrically 30 min after treatment with the poison. Group B animals were administered CrO_3 at LD_{50} once intragastrically. NaMSA at a dose of 100 mg/kg b.w. was administered intragastrically 30 min after treatment with the poison.

NaMSA was administered intragastrically shortly after poisoning because the aim of this stage of the study was to evaluate the efficacy of NaMSA as a "local" antidote inhibiting chromium absorption from the gastrointestinal tract by altering its oxidation state.

Group C animals were administered CrO_3 at LD_{50} once intragastrically. NaMSA at a dose of 50 mg/kg b.w. was administered intraperitoneally two hours after treatment with the poison and then twice a day (at 8 a.m. and 2 p.m.) for four days. Group D animals were administered CrO_3 at LD_{50} once intragastrically. NaMSA at a dose of 100 mg/kg b.w. was administered intraperitoneally two hours after treatment with the poison and then twice a day (at 8 a.m. and 2 p.m.) for four days.

Chromium absorption from the gastrointestinal tract is gradual, since a part of the Cr(VI) ions are reduced in the presence of hydrochloric acid to Cr(III), which is absorbed much more poorly [13]. The elimination of hexavalent chromium from the serum in rats has one phase, elimination from the liver is triphasic with three biological half-lives (2.4 h, 52.8 h, and 15.7 days), and from the kidney this element is eliminated in two phases with two biological half-lives (52.8 h and 10.5 days) [14]. For this reason, NaMSA was administered not only on the day of poisoning, but also for the four following days.

Intragastric administration was performed using a metal tube. CrO_3 was applied intragastrically after dissolving it in 0.9% NaCl in a volume of 5 ml/kg rat weight. NaMSA was dissolved in 0.9% NaCl and administered intragastrically (groups A and B) or intraperitoneally (groups C and D) in a volume of 10 ml/kg. The animals were deprived of food for 16 h before treatment with the test substances. They were given access to food again 7 h after poisoning [11].

Blood was drawn from the tail vein of all the rats before the observation began (day 0) and on days 7 and 14 to determine indicators of liver and kidney function in the serum (activity of aminotransferases and the concentrations of bilirubin, urea, and creatinine). Blood for evaluation of serum chromium concentration was collected before poisoning (day 0) and after the observation ended (day 14). The livers and kidneys of animals which died and from the remaining animals after completing the observation were collected for the determination of chromium concentrations in these organs. Tissue were subjected to "wet" mineralization to obtain a homogenous solution. Chromium concentration in the blood and internal organs after mineralization was measured by atomic absorption spectrometry (SOLAAR M6 Spectrometer, ThermoElemental). Animals which died were examined at autopsy and their organs were evaluated under a microscope.

After the experiments were completed, the mortality, biochemical parameters (Dimension Panda Analyzer, DADE Behring), and chromium concentrations in the test groups in comparison with the control group were analyzed.

Statistical Analysis

The results are presented as the means ± SD. Non-parametric values (mortality) were compared using the χ^2 test, whereas differences between parametric values (results of biochemical tests and chromium concentrations) were analyzed by individual comparison with one-way ANOVA. Initially, the normal distribution of all parametric data was tested by the Shapiro-Wilk's test [15]. Statistical analysis was carried out using Statistica software.

Results

Analysis of Mortality

The greatest loss of animals occurred in the first week, particularly during the first three days of observation in all groups, i.e. the control group and the test groups. Only intragastric administration of NaMSA at 100 mg/kg (group B) decreased mortality in acute CrO_3 poisoning in rats. There were no differences in mortality between males and females in all groups.

Analysis of Biochemical Parameters

The activities of aminotransferases and the bilirubin concentration in blood were observed to increased in groups K, C, and D. In these groups, elevations in blood urea and blood creatinine concentrations were also observed, which indicates damage to the parenchyma of the liver and kidneys. Better values of the biochemical indicators of liver and kidney damage were noted in groups A and B than in the controls.

Analysis of Blood Chromium Concentrations

Only the group of rats which received intragastrically 100 mg/kg dose of NaMSA (group B, Table 5) showed no increase in blood chromium

concentration on day 14 of the study compared with the values before the experiments started. Also in group B, the blood chromium concentration on day 14 was statistically significantly lower than in the control group K (Table 6).

Analysis of Chromium Concentrations in the Liver and Kidneys

The increase in chromium concentrations in the liver and kidneys was the weakest in rats treated intragastrically with NaMSA (groups A and B).

Table 1. Mortality

Tabela 1. Śmiertelność

Group (Grupa)	K (n = 20)	A (n = 20)	B (n = 20)	C (n = 20)	D (n = 20)
DN	12	9	4	11	10
%	60	45	20	55	50
χ^2	–	0.902	6.67	0.102	0.404
p	–	NS	≤ 0.01	NS	NS

n – number of animals per group,
DN – number of animals died per group,
% – percent of animals died per group,
p – vs. control group.

n – liczba zwierząt w grupie,
DN – liczba padłych zwierząt w grupie,
% – odsetek padłych zwierząt w grupie,
p – w porównaniu do grupy kontrolnej.

It should be noted that CrO_3 poisoning caused a much greater rise in chromium concentration in the internal organs than in the blood.

Analysis of Autopsy Results and Histopathological Examination

All animals which died had swollen stomachs and bowel loops. They showed macroscopic signs of intense hemorrhagic gastroenteritis. Gastric perforation was noticed in one animal from group K and in one animal from group D. Perforation of the gastrointestinal tract walls was not observed in animals of groups A, B, and C; moreover, signs of hemorrhagic gastroenteritis were the weakest in group B.

Histopathological examination of the liver specimens from the rats of all the groups demonstrated marked venous congestion, particularly in the area of the central vein. Congestion intensity varied, but the smallest was seen in group B. Some preparations showed features of slight microvacuole steatosis. The intensity of these changes did not depend on the group to which a rat belonged. No other significant morphological changes, in particular no coagulative necrosis of the intermediate zone of the liver lobule characteristic of acute chromium poisoning, were observed in the examined specimens.

Histopathological examination of the kidney specimens from rats of all groups showed venous

Table 2. Analysis of biochemical parameters in groups K, A, B, C, D on day 0 (mean values)

Tabela 2. Analiza wskaźników biochemicznych w grupach K, A, B, C, D w dniu 0 (wartości średnie)

Group (Grupa)	Urea (Mocznik) mmol/l	Creatinine (Kreatynina) µmol/l	Billirubin (Bilirubina) µmol/l	AlAT U/l	AspAT U/l
K (n = 20)	7.16 ± 1.01	58.5 ± 6.59	2.01 ± 0.40	101.9 ± 6.01	66.85 ± 7.36
%	100	100	100	100	100
A (n = 20)	7.28 ± 1.09	61.3 ± 8.43	1.93 ± 0.41	104.7 ± 9.0	67.25 ± 7.53
%	101.61	104.79	95.78	102.75	100.59
p	NS	NS	NS	NS	NS
B (n = 20)	7.158 ± 8.2	59.75 ± 6.57	1.92 ± 0.44	107.9 ± 14.37	65.05 ± 9.80
%	99.89	102.14	95.28	105.88	97.3
p	NS	NS	NS	NS	NS
C (n = 20)	7.254 ± 1.34	59.55 ± 10.34	2.07 ± 0.67	107.35 ± 14.42	65.2 ± 8.46
%	101.23	101.79	102.73	105.35	97.53
p	NS	NS	NS	NS	NS
D (n = 20)	7.349 ± 1.06	60.9 ± 6.62	1.97 ± 0.43	105.45 ± 9.35	63.35 ± 7.87
%	102.55	104.10	97.76	103.48	94.76
p	NS	NS	NS	NS	NS

n – number of animals,
p – vs. control group.

n – liczba zwierząt,
p – w porównaniu do grupy kontrolnej.

Table 3. Analysis of biochemical parameters in groups K, A, B, C, D on day 7 (mean values)

Tabela 3. Analiza wskaźników biochemicznych w grupach K, A, B, C, D w dniu 7 (wartości średnie)

Group (Grupa)	Urea (Mocznik) mmol/l	Creatinine (Kreatynina) µmol/l	Billirubin (Bilirubina) µmol/l	AlAT U/l	AspAT U/l
K (n = 10) % 	10.92 ± 1.21 100 	70.8 ± 4.91 100 	3.89 ± 0.56 100 	231.6 ± 32.14 100 	114.7 ± 14.69 100
A (n = 12) % *p*	8.36 ± 1.35 76.54 ≤ 0.001	70.5 ± 5.84 99.57 NS	2.58 ± 0.60 66.41 ≤ 0.001	178.75 ± 14.24 77.18 ≤ 0.001	97.33 ± 13.09 84.86 ≤ 0.01
B (n = 16) % *p*	7.47 ± 1.22 68.45 ≤ 0.001	63.81 ± 5.96 90.13 ≤ 0.005	2.31 ± 0.52 59.45 ≤ 0.001	117.0 ± 16.30 50.52 ≤ 0.001	78.5 ± 11.83 68.44 ≤ 0.001
C (n = 10) % *p*	10.68 ± 1.11 97.80 NS	72.9 ± 5.88 102.97 NS	3.57 ± 0.49 91.77 NS	190.0 ± 69.34 82.04 NS	116.5 ± 14.40 101.57 NS
D (n = 12) % *p*	9.93 ± 2.16 90.96 NS	74.83 ± 11.10 105.69 NS	3.62 ± 0.46 93.19 NS	218.42 ± 21.63 94.31 NS	119.67 ± 15.24 104.33 NS

n – number of animals,
p – vs. control group.

n – liczba zwierząt,
p – w porównaniu do grupy kontrolnej.

Table 4. Analysis of biochemical parameters in groups K, A, B, C, D on day 14 (mean values)

Tabela 4. Analiza wskaźników biochemicznych w grupach K, A, B, C, D w dniu 14 (wartości średnie)

Group (Grupa)	Urea (Mocznik) mmol/l	Creatinine (Kreatynina) µmol/l	Billirubin (Bilirubina) µmol/l	AlAT U/l	AspAT U/l
K (n = 8) % 	10.64 ± 1.36 100 	71.37 ± 6.52 100 	4.24 ± 0.39 100 	284.5 ± 36.28 100 	114.62 ± 14.14 100
A (n = 11) % *p*	8.08 ± 1.44 75.97 ≤ 0.005	67.73 ± 5.59 94.89 NS	2.98 ± 0.70 70.37 ≤ 0.001	197.18 ± 11.13 69.31 ≤ 0.001	97.27 ± 10.18 84.86 ≤ 0.001
B (n = 16) % *p*	6.98 ± 1.12 65.63 ≤ 0.001	64.12 ± 6.32 89.84 ≤ 0.02	2.82 ± 0.53 66.52 ≤ 0.001	123.44 ± 19.40 43.38 ≤ 0.001	69.56 ± 7.95 60.69 ≤ 0.001
C (n = 9) % *p*	9.88 ± 1.34 92.86 NS	77.44 ± 7.79 108.50 NS	4.13 ± 0.41 97.54 NS	244.56 ± 57.24 85.96 NS	124.55 ± 16.06 108.66 NS
D (n = 10) % *p*	9.62 ± 2.59 90.43 NS	70.2 ± 6.49 98.35 NS	4.13 ± 0.48 97.46 NS	282.9 ± 36.77 99.44 NS	117.3 ± 16.94 102.33 NS

n – number of animals,
p – vs. control group.

n – liczba zwierząt,
p – w porównaniu do grupy kontrolnej.

congestion of various intensity. The most serious microscopic changes were observed in the kidneys of rats from groups K, C, and D, with signs of marked necrosis, particularly in the area of the proximal tubules. The contour of the tubule was preserved, but only a zone of the formerly present epithelium was visible; however, the nuclei could not be distinguished. The epithelium of the non-necrotic tubules was delaminated. Necrosis of the proximal tubules was not observed in animals of groups A and B. However, tubular epithelial delamination was noted, similar to that in the control group. The smallest morphological changes were seen in the group of rats which were given

Table 5. Blood chromium concentrations in rats of groups K, A, B, C, D on day 14 compared with those on day 0

Tabela 5. Stężenie chromu we krwi szczurów z grup K, A, B, C, D w dniu 14 w porównaniu do stężenia chromu we krwi w dniu 0

Blood chromium concentration (Stężenie chromu we krwi szczurów) µg/l				
Group (Grupa)	day 0		day 14	
K	X (n = 20) % *p*	0.992 ± 0.101 100 –	X (n = 8) % *p*	1.898 ± 0.194 191.28 ≤ 0.001
A	X (n = 20) % *p*	1.010 ± 0.129 100 –	X (n = 11) % *p*	1.879 ± 0.332 186.05 ≤ 0.001
B	X (n = 20) % *p*	0.960 ± 0.071 100 –	X (n = 16) % *p*	0.976 ± 0.064 101.62 NS
C	X (n = 20) % *p*	0.978 ± 0.135 100 –	X (n = 9) % *p*	1.951 ± 0.174 199.5 ≤ 0.001
D	X (n = 20) % p	0.976 ± 0.178 100 –	X (n = 10) % p	1.931 ± 0.195 197.74 ≤ 0.001

n – number of animals,
X – mean blood chromium concentration,
p – vs. day 0.

n – liczba zwierząt,
X – średnie stężenie chromu we krwi,
p – w porównaniu do dnia 0.

Table 6. Blood chromium concentrations in rats of the test groups A, B, C, D compared with those of rats of the control group K

Tabela 6. Stężenie chromu we krwi szczurów grup badanych A, B, C, D w porównaniu do stężenia chromu we krwi szczurów grupy kontrolnej K

Blood chromium concentration (Stężenie chromu we krwi szczurów) µg/l				
Group (Grupa)	day 0		day 14	
K	X (n = 20) %	0.992 ± 0.101 100	X (n = 8) %	1.898 ± 0.194 100
A	X (n = 20) % *p*	1.010 ± 0.129 101.81 NS	X (n = 11) % *p*	1.879 ± 0.332 99.03 NS
B	X (n = 20) % *p*	0.960 ± 0.071 96.77 NS	X (n = 16) % *p*	0.976 ± 0.064 51.42 ≤ 0.001
C	X (n = 20) % *p*	0.978 ± 0.135 98.59 NS	X (n = 9) % *p*	1.951 ± 0.174 102.82 NS
D	X (n = 20) % *p*	0.976 ± 0.178 98.44 NS	X (n = 10) % *p*	1.931 ± 0.195 101.76 NS

n – number of animals,
X – mean chromium concentration,
p – vs. group K.

n – liczba zwierząt,
X – średnie stężenie chromu,
p – w porównaniu do grupy K.

Table 7. Chromium concentrations in the internal organs of rats of the test groups A, B, C, D compared with those of rats of the control group K

Tabela 7. Stężenie chromu w narządach wewnętrznych szczurów grup badanych A, B, C, D w porównaniu ze stężeniami chromu w narządach wewnętrznych szczurów grupy kontrolnej K

Chromium concentration in internal organs of rats – μg/100 g of tissue (Stężenie chromu w narządach wewnętrznych szczurów – μg/100 g tkanki)				
Group (Grupa)	liver		kidney	
K	X (n = 20) % 	72.95 ± 36.76 100 	Kx (n = 20) % 	496.05 ± 276.74 100
A	X (n = 20) % *p*	20.1 ± 8.29 27.55 ≤ 0.001	Ax (n = 20) % *p*	169.7 ± 66.05 34.21 ≤ 0.001
B	X (n = 20) % *p*	6.85 ± 2.06 9.36 ≤ 0.001	Bx (n = 20) % *p*	36.9 ± 14.32 7.44 ≤ 0.001
C	X (n = 20) % *p*	75.2 ± 32.79 103.08 NS	Cx (n = 20) % *p*	492.3 ± 269.1 99.24 NS
D	X (n = 20) % *p*	77.65 ± 39.96 106.44 NS	Dx (n = 20) % *p*	507.75 ± 282.24 102.36 NS

n – number of animals,
X – mean chromium concentration,
p – vs. group K.

n – liczba zwierząt,
X – średnie stężenie chromu,
p – w porównaniu do grupy K.

NaMSA intragastrically at the dose of 100 mg/kg (group B). Only trace tubular epithelial delamination could be noticed in this group. No morphological changes in the renal glomerules ensued in any of the groups.

Discussion

The greatest loss of animals occurred in the first week, particularly within the first three days of observation. Hence it can be assumed that the deaths were not the result of damage to internal organs, but caused by the direct caustic action of Cr(VI) ions on the digestive tract walls. Cr(VI) ions exhibit a very strong caustic and irritating action, leading to severe hemorrhagic gastroenteritis with consequent dehydration and circulatory failure. This was confirmed by examination at autopsy, since all the animals which died had serious hemorrhagic gastroenteritis, complicated in two cases by gastric perforation. The extensive passive congestion of the internal organs (liver and kidney) demonstrated by histopathological examination also indirectly suggests that animals' deaths were caused by circulatory insufficiency and not by internal organ damage. These suppositions are in agreement with observations by other authors [16]. Theoretically, the absorption of hexavalent chromium ions from the digestive tract in rats was estimated at 2–5% [13]; however, the application of a high dose of CrO_3 in the present study caused a significant rise in blood chromium concentrations in groups K, A, C, and D (Table 5). The elevated chromium absorption from the digestive tract was possibly due to Cr(VI)-induced damage to the intestinal barrier.

The activities of aminotransferases and the bilirubin concentration in the blood were observed to increase in groups K, C, and D, which indicates hepatocyte damage, but there were no signs of coagulative necrosis of the lobule intermediate zone, although the latter is considered to be a characteristic morphological feature of chromium-induced liver damage [4, 16]. Therefore it can be assumed that the liver damage was not strong enough to cause the death of the animals.

In groups K, C, and D there was chromium accumulation in the kidneys and elevations in the blood content of urea and creatinine. However, microscopic examination did not reveal pathological changes in renal glomerules. Hence it seems that the retention of urea and creatinine was not caused by renal parenchyma damage, but by dehy-

dration following severe gastroenteritis. The proximal tubule necrosis observed in groups K, C, and D was similar as in other papers on acute chromium poisoning [7, 16].

The treatment of acute CrO_3 poisoning with NaMSA was carried out in two different ways: NaMSA was applied as a "local" antidote (groups A and B) and as a systemically acting antidote (groups C and D).

Lowered mortality, which is the most important indicator of treatment efficiency, was observed only after intragastrical NaMSA administration at 100 mg/kg (group B). Furthermore, hemorrhagic gastroenteritis was the mildest in this group. Intragastric administration of NaMSA at 100 mg/kg suppressed absorption of Cr(VI) ions from the digestive tract, resulting in the lack of a significant increase in blood chromium concentration (Tables 5, 6), a much lower chromium concentration in the internal organs in comparison with the control group (Table 7), and better values of the biochemical indicators of liver and kidney damage than in the controls (Tables 2–4).

Diminution of chromium absorption from the gastrointestinal tract was also observed in the group of rats which received the 50 mg/kg intragastric dose of NaMSA (group A). Although the increase in blood chromium concentration in this group was marked, chromium content in the internal organs was significantly lower than in the control group (Table 7), while the biochemical indicators of liver and kidney damage were also lower than in the control group (Tables 2–4). However, it should be emphasized that mortality in group A was not reduced from that of controls (Table 1). Hence it should be concluded that treatment of acute CrO_3 poisoning with NaMSA at 50 mg/kg was inefficient and that the loss of animals resulted from the direct effect of Cr(VI) ions on the digestive tract walls.

Intraperitoneal NaMSA administration (groups C and D) was performed in order to reduce the amount of Cr(VI) ions absorbed to the circulation, since it is known that cells are damaged when haxavalent ions enter the cytoplasm and are intracellularly reduced to trivalent ions [1]. However, treatment of acute CrO_3 poisoning with intraperitoneal NaMSA administration (groups C and D) proved completely ineffective. There was no reduction in mortality (Table 1) in these groups, whereas the chromium concentrations in the blood and internal organs were close to control values (Tables 6, 7) and the biochemical indicators of internal organ damage did not differ significantly from those of the control group (Tables 2–4).

NaMSA was shown in an *in vitro* study to strongly reduce Cr(VI) ions to Cr(III) ions [17]. The absorption of Cr(III) ions is many times weaker than that of Cr(VI) ions; in addition, Cr(III) ions, in contrast to Cr(VI) ions, have practically no caustic and irritative properties [1]. The optimal conditions for Cr(VI) reduction by NaMSA were demonstrated to be 40°C (313 K), pH 1–4, and 2 h [17]. Obviously, after intragastric NaMSA administration to rats, only two of these conditions are present, since the gastric contents leave the stomach within a much shorter time than 2 h. On the other hand, after a caustic substance is introduced into the stomach, reflex pylorus constriction can occur, which would prolong the transit of the gastric contents, thereby allowing for the reduction of a considerable number of Cr(VI) ions.

In vitro studies have also revealed that NaMSA is able to form complexes with chromium ions, but only with Cr(III) ions [17], which suggests that it was not the formation of complexes, but the reduction of Cr(VI) that was the main detoxifying action of NaMSA.

Acknowledgements. The experiments were performed after approval by the Local Ethics Commission for Experiments on Animals in Wrocław (license no. 4/05). The study was supported by budgetary funds of the Department of Pharmacology, Silesian Piasts University of Medicine in Wrocław.

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Conflict of interest: None declared

Received: 14.04.2006
Revised: 28.06.2006
Accepted: 21.09.2006

Praca wpłynęła do Redakcji: 14.04.2006 r.
Po recenzji: 28.06.2006 r.
Zaakceptowano do druku: 21.09.2006 r.