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JAN MAGDALAN[1], ADAM SZELĄG[1], MARIA KOPACZ[2], ANNA KUŹNIAR[2], DOROTA NOWAK[2], PRZEMYSŁAW KOWALSKI[3], MAŁGORZATA PIEŚNIEWSKA[1]

Quercetin-5'-Sulfonic Acid Sodium Salt and Morin-5'-Sulfonic Acid Sodium Salt as Antidotes in the Treatment of Acute Inorganic Mercury Poisoning – Experimental Studies

Sól sodowa kwasu kwercetyno-5'-sulfonowego i kwasu moryno-5'-sulfonowego jako odtrutki w leczeniu ostrego zatrucia nieorganicznym związkiem rtęci – badania doświadczalne

[1] Department of Pharmacology, Silesian Piasts University of Medicine in Wrocław, Poland
[2] Department of Inorganic and Analytical Chemistry, Chemical Faculty, University of Technology, Rzeszów, Poland
[3] Department of Pathological Anatomy, Silesian Piasts University of Medicine in Wrocław, Poland

Abstract

Background. Acute poisoning with inorganic mercury is associated with high mortality. There is no antidote, which administered orally would bind mercury ions in the digestive tract and suppress its absorption to systemic circulation. *In vitro* studies have demonstrated that quercetin-5'-sulfonic acid sodium salt (NaQSA) and morin-5'-sulfonic acid sodium salt (NaMSA) form complexes with mercury. Therefore, it can be expected that oral administration of NaQSA and NaMSA in mercury poisoning will promote formation of their insoluble complexes with mercury ions in the digestive tract, thereby lowering mercury absorption from the gastrointestinal tract to systemic circulation which will prevent poisoning or alleviate its course.
Objectives. Determining the therapeutic efficacy of intragastrical NaQSA and NaMSA administration in acute mercuric chloride ($HgCl_2$) poisoning.
Material and Methods. Wistar rats, divided in control group (K) and test groups (A, B, C, D), were poisoned with a single intragastrical administration of $HgCl_2$ at its LD_{50}. Animals in the group K received no treatment, while the test groups were treated intragastrically 30 min after poisoning with the following single doses of the antidotes: group A – NaQSA 50 mg/kg, group B – NaQSA 100 mg/kg, group C – NaMSA 50 mg/kg, group D – NaMSA 100 mg/kg.
Results. The mortality was lower and biochemical indices of renal damage were more satisfactory in groups A, B and D in comparison with group K. In all tested groups NaQSA and NaMSA diminished mercury absorption and renal mercury accumulation.
Conclusions. Intragastrical NaQSA administration at a single dose of 50 or 100 mg/kg and single intragastrical NaMSA treatment at 100 mg/kg lowered the mortality of rats poisoned acutely with an inorganic mercury compound – $HgCl_2$. The mechanism of detoxifying action of NaQSA and NaMSA is most probably based on their ability to form insoluble complexes with mercury ions, which are not absorbed from the digestive tract and are excreted in feces (**Adv Clin Exp Med 2006, 15, 4, 581–587**).

Key words: mercuric chloride, acute poisoning, NaQSA, NaMSA, rats.

Streszczenie

Wprowadzenie. Ostre zatrucie nieorganicznymi związkami rtęci charakteryzuje się dużą śmiertelnością. Dotychczas nie dysponowano skuteczną odtrutką, która po podaniu doustnym mogłaby związać jony rtęci w przewodzie pokarmowym i zahamować ich wchłanianie. Badania *in vitro* wykazały, że sól sodowa kwasu kwercetyno-5'-sulfonowego (NaQSA) i sól sodowa kwasu moryno-5'-sulfonowego (NaMSA) tworzą z rtęcią nierozpuszczalne kompleksy, dlatego można przypuszczać, iż doustne podanie tych substancji jako „odtrutek miejscowych" mogłoby zahamować wchłanianie rtęci z przewodu pokarmowego.

Cel pracy. Określenie skuteczności leczenia ostrego zatrucia chlorkiem rtęciowym ($HgCl_2$) za pomocą dożołądkowego podania NaQSA lub NaMSA.
Materiał i metody. U szczurów szczepu Wistar podzielonych na grupę kontrolną (K) i grupy badane (A, B, C, D) wywołano ostre zatrucie, podając $HgCl_2$ w dawce odpowiadającej LD_{50}. Zwierzęta z grupy K nie otrzymały leczenia, w grupach badanych natomiast 30 min po zastosowaniu trucizny podano dożołądkowo badane odtrutki w następujących dawkach jednorazowych: grupa A – NaQSA 50 mg/kg m.c., grupa B – NaQSA 100 mg/kg m.c., grupa C – NaMSA 50 mg/kg m.c., grupa D – NaMSA 100 mg/kg m.c.
Wyniki. Śmiertelność oraz biochemiczne wykładniki uszkodzenia nerek były znacznie niższe w grupach A, B i D w porównaniu do grupy K. We wszystkich grupach badanych podanie NaQSA i NaMSA zmniejszyło wchłanianie rtęci z przewodu pokarmowego i gromadzenie się tego pierwiastka w nerkach.
Wnioski. Dożołądkowe podanie NaQSA w jednorazowej dawce 50 lub 100 mg/kg oraz jednorazowe dożołądkowe podanie NaMSA w dawce 100 mg/kg zmniejszyło śmiertelność szczurów ostro zatrutych nieorganicznym związkiem rtęci – $HgCl_2$. Mechanizm działania NaQSA i NaMSA polega prawdopodobnie na tworzeniu z jonami rtęci nierozpuszczalnych kompleksów, które nie wchłaniają się z przewodu pokarmowego i zostają wydalone z kałem (**Adv Clin Exp Med 2006, 15, 4, 581–587**).

Słowa kluczowe: chlorek rtęciowy, ostre zatrucie, NaQSA, NaMSA, szczury.

Acute oral administration of inorganic mercury compounds leads to hemorrhagic gastroenteritis followed by dehydration and circulatory failure. Patients who survive the first stage of poisoning frequently suffer renal damage and rarer injury of other parenchymal organs [1–3]. Vomiting and acute abdominal pain appear as early as 10 – 15 min after ingestion of inorganic mercury compounds [1, 3], so patients are usually relatively quickly admitted to a hospital. Unfortunately, due to caustic action of these compounds, attempts to eliminate the poison from the gastrointestinal tract, e.g. by gastric lavage, is often contraindicated. There is no efficient antidote, which administered orally would bind mercury ions in the gastrointestinal tract and suppress their absorption to systemic circulation. It is recommended to drink milk or egg white to promote formation of insoluble proteinates [2], however, efficacy of such intervention has not been proven. Some chelating agents, e.g. d-penicillamine and meso-2,3-dimercaptosuccinic acid can be administered orally [2]. Nevertheless, these substances are not used as "local" antidotes for purpose of mercury binding in the digestive tract, but they are applied to complex mercury ions that had already been absorbed to the systemic circulation.

In vitro studies have indicated that some compounds belonging to polyhydroxyflavones, e.g. morin (3,5,7,2',4'-pentahydroxyflavone) and quercetin (3,5,7,3',4'-pentahydroxyflavone) and their sulfonic derivatives characterized by good aqueous solubility, like quercetin-5'-sulfonic acid sodium salt (NaQSA) and morin-5'-sulfonic acid sodium salt (NaMSA) form complexes with mercury and cadmium. These complexes are poorly soluble in water and are formed in the environment with acidic pH (1.3–1.7) like that occurring in the stomach [4]. Therefore, it can be expected that oral administration of NaQSA and NaMSA in mercury poisoning will promote formation of their insoluble complexes with mercury ions in the digestive tract, thereby lowering mercury absorption from the gastrointestinal tract to systemic circulation which will prevent poisoning or alleviate its course.

The aim of the present study was to assess the efficacy of NaQSA and NaMSA as gastrointestinal-local antidotes in the treatment of experimental acute poisoning with an inorganic mercury compound (mercuric chloride – $HgCl_2$) in rats. We evaluated the effect of NaQSA and NaMSA treatment on mortality, biochemical indices of nephrotoxicity, mercury content in the kidneys and the microscopic picture of renal tissue.

Material and Methods

Animals

The study was conducted on 100 Wistar rats of both sexes with body weight averaging 200 ± 16.8 g. Animals were housed under standard conditions (lights on from 07:00 to 21:00 and temperature 22 ± 2°C) with water and granulated food (LSM, "Agropol", Motycz) available *ad libitum*.

Chemicals

Acute poisoning with an inorganic mercury compound was elicited by administration of $HgCl_2$ to animals (Polish Reagents Co., cat. no. czda-768670117). $HgCl_2$ was chosen because of its very good solubility in water (7.4 g/100 g). The poison was administered intragastrically at its LD_{50} which in rats was estimated at 37 mg/kg [5].

The sulfonic quercetin derivative NaQSA and the sulfonic morin derivative NaMSA were used as antidotes. These substances are characterized by low toxicity to laboratory animals. Both substances were applied at doses of 50 and 100 mg/kg

b.w., which correspond to 1/40 and 1/20 of their LD_{50} in rats [6, 7].

NaQSA and NaMSA were synthesized according to the methods described previously [8]. The purity of the obtained compounds was checked with thin-layer chromatography on alumina plates covered with an adsorbent (silica gel 60 WF_{254}, MERCK) using a solvent system n-butanol – acetic acid – water (4 : 1 : 5). It was demonstrated that the compounds NaQSA and NaMSA were homogenous substances and did not contain any untransformed substrates. Molecular composition of the products was confirmed by elemental analysis of C, H, S, determination of the number of crystalline water molecules by gravimetric and derivatographic method, and sodium content was established by atomic absorption spectrometry. Spectrophotometric characteristics of the NaQSA and NaMSA were found to be concordant with literature data [8].

NaQSA (Fig. 1) and NaMSA (Fig. 2) are easily soluble in water and keep properties of the parent compounds. The aqueous solubility of NaQSA at 22°C ± 1°C (295 K) was estimated at $5.0 \cdot 10^{-3}$ mol/dm^3, while the aqueous solubility of NaMSA under the same conditions was $2.7 \cdot 10^{-2}$ mol/dm^3. Sulfonic quercetin and morin derivatives can be considered to be multiprotonic acids, which dissociate in aqueous solutions yielding respective anions. Their dissociation constants (pK_a) in aqueous solution determined at 20°C and I = 0.1 by potentiometric method were: pK= 4.67; pK= 7.84; pK= 9.82; pK= 10.69 for NaMSA, and pK= 7.43; pK= 8.16; pK= 9.24; pK= 10.84 for NaQSA [8, 9].

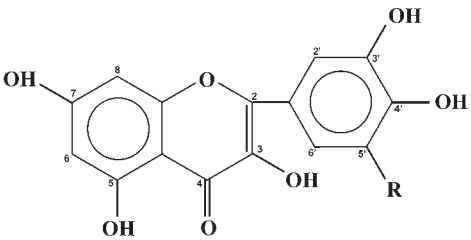

Fig. 1. Structural formula of quercetin (R = H) and NaQSA (R = SO_3Na)

Ryc. 1. Wzór strukturalny kwercetyny (R = H) i NaQSA (R = SO_3Na)

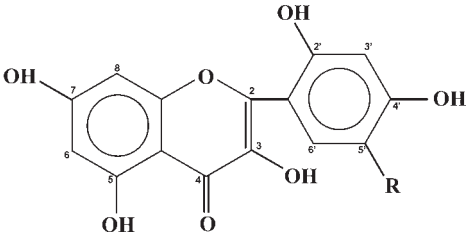

Fig. 2. Structural formula of morin (R = H) and NaMSA (R = SO_3Na)

Ryc. 2. Wzór strukturalny moryny (R = H) i NaMSA (R = SO_3Na)

Experimental Procedures

Rats were divided into control group (K) and test groups (A, B, C, D). Each group comprised 20 animals half of which were females and another half were males. The animals were observed for 14 days after poisoning [10]. All rats were poisoned with mercury by a single intragastrical administration of $HgCl_2$ at its LD_{50}. Animals belonging to the control group did not receive any treatment while rats from the test groups were intragastrically administered 30 min after poisoning with the following single doses of the antidotes: group A – 50 mg/kg b.w. NaQSA; group B – 100 mg/kg b.w. NaQSA; group C – 50 mg/kg b.w. NaMSA; group D – 100 mg/kg b.w. NaMSA.

The substances were dissolved in 0.9% NaCl and given intragastrically using a metal tube. $HgCl_2$ was administered in 5 ml/kg volume whereas the treatment volume of NaQSA and NaMSA was 10 ml/kg. The animals were starved for 16 hours prior to the treatment with test substances and were given free access to food again 4 hours after poison administration [10].

Before administration of the poison (day "0") and then on day 7 and 14 after administration of the poison, blood was collected from the tail vein for determination of serum levels of renal function indicators (urea and creatinine).

After the end of the observation or animals' death, the kidneys were collected for evaluation of renal mercury concentration. The tissue was mineralized, and mercury content was assayed by atomic absorption spectrometry. All animals were examined at autopsy, and their kidneys were subjected to microscopic evaluation.

When the experiment was finished, mortality, biochemical parameters of renal function and mercury levels in the test groups were compared with respective values in the control group.

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 mercury concentrations) were analyzed by individual comparison with the one-way ANOVA. Initially, normal distribution of all parametric data was tested by the Shapiro-Wilk's test [11]. Statistical analysis was carried out by the use of Statistica software.

Results

Analysis of Mortality

There were no differences in mortality between males and females within any group (Table 1).

Analysis of Biochemical Indicators of Renal Function and Mercury Concentrations in the Kidney

See Tables 2–5.

Analysis of Autopsy Results and Histopathological Studies

All animals exhibited macroscopic signs of hemorrhagic gastroenteritis, i.e. erosions and extravasations of the gastrointestinal tract walls. Gastroenteritis symptoms were the gravest in group K and C, its moderate signs were observed in group A and D whereas the weakest gastroenteritis symptoms were seen in group B. There were no cases of digestive tract perforation.

Animals from the control group which died in the course of the experiment exhibited signs of massive necrosis of proximal and distal renal tubules with subsequent accumulation of calcium salts in tubular walls. In the remaining control animals, no calcium salt accumulation occurred, but delamination of the tubular epithelium resulting from cell membrane damage was observed, however, the nuclei were preserved.

Table 2. Biochemical indicators of renal function in group K (mean value)

Tabela 2. Biochemiczne wskaźniki funkcji nerek w grupie K (wartości średnie)

Day of the experiment (Dzień badania)	Urea (Mocznik) [mmol/L]	Creatinine (Kreatynina) [µmol/L]
„0" (n = 20)	6.92 ± 1.14	61.2 ± 12.47
%	100	100
„7" (n = 9)	21.56 ± 7.37	200.1 ± 176.92
%	311.3	326.97
p	≤ 0.001	≤ 0.005
„14" (n = 8)	31.5 ± 11.49	262.37 ± 210.35
%	454.9	428.71
p	≤ 0.001	≤ 0.001

p – in comparison to the day 0.

p – w porównaniu z dniem 0.

In the treatment groups, structural renal changes were observed only in animals which died during the observation period. These changes were noticed in proximal tubules and were caused by cell membrane damage followed by epithelium delamination towards tubule lumen. Nuclei were intact and no saturation with calcium salts was seen in the tubules. There were no glomerular changes in any group.

Discussion

Acute poisoning with inorganic mercury compounds, e.g. sublimate, is associated principally with gastrointestinal and renal damage (Table 2). Gastrointestinal tract walls are lesioned by direct caustic action of inorganic mercury compounds,

Table 1. Mortality

Tabela 1. Śmiertelność

Group (Grupa)	K (n = 20)	A (n = 20) 50 mg/kg NaQSA	B (n = 20) 100 mg/kg NaQSA	C (n = 20) 50 mg/kg NaMSA	D (n = 20) 100 mg/kg NaMSA
D	12	3	2	9	4
%	60	15	10	45	20
χ^2	–	8.64	10.99	0.90	6.66
p	–	≤ 0.01	≤ 0.005	NS	≤0.001

n – number of animals in the group.
D – number of dead animals in the group.
% – percent of dead animals in the group.
p – in comparison with the control group.

n – liczba zwierząt w grupie.
D – liczba padłych zwierząt w grupie.
% – odsetek padłych zwierząt w grupie.
p – w porównaniu z grupą kontrolną.

Table 3. Blood urea concentration on days 0, 7, 14 (mean value)

Tabela 3. Stężenie mocznika we krwi w dniach 0, 7, 14 (wartości średnie)

Group (Grupa)	Urea (Mocznik) mmol/l		
Day (Dzień)	"0"	"7"	"14"
K (n = 20) %	6.92 ± 1.14 100	21.56 ± 7.37 100	31.5 ± 11.49 100
A (n = 20) 50 mg/kg NaQSA % p	 6.82 ± 1.13 98.56 NS	 7.87 ± 1.13 36.50 ≤ 0.001	 8.17 ± 1.74 25.96 ≤ 0.001
B (n = 20) 100 mg/kg NaQSA % p	 6.75 ± 1.38 97.47 NS	 7.63 ± 1.38 35.4 ≤ 0.001	 7.36 ± 1.28 23.37 ≤ 0.001
C (n = 20) 50 mg/kg NaMSA % p	 6.45 ± 1.58 93.14 NS	 18.29 ± 6.54 84.83 NS	 31.64 ± 7.94 100.43 NS
D (n = 20) 100 mg/kg NaMSA % p	 6.60 ± 1.54 95.30 NS	 8.47 ± 2.29 39.29 ≤ 0.001	 9.18 ± 1.42 29.17 ≤ 0.001

n – number of animals.
p – in comparison with the control group.

n – liczba zwierząt.
p – w porównaniu z grupą kontrolną.

Table 4. Blood creatinine concentration on days 0, 7, 14 (mean value)

Tabela 4. Stężenie kreatyniny we krwi w dniach 0, 7, 14 (wartości średnie)

Group (Grupa)	Creatinine (Kreatynina) µmol/l		
Day (Dzień)	"0"	"7"	"14"
K (n = 20) %	61.2 ± 12.47 100	200.1 ± 176.92 100	262.37 ± 210.35 100
A (n = 20) 50 mg/kg NaQSA % p	 61.1 ± 12.23 99.83 NS	 69.05 ± 14.93 34.507 ≤ 0.005	 69.0 ± 11.32 26.29 ≤ 0.001
B (n = 20) 100 mg/kg NaQSA % p	 61.55 ± 0.07 100.57 NS	 65.1 ± 10.86 32.53 ≤ 0.005	 70.17 ± 11.69 26.74 ≤ 0.001
C (n = 20) 50 mg/kg NaMSA % p	 59.80 ± 11.10 97.71 NS	 122.79 ± 44.92 61.36 NS	 208.0 ± 78.75 79.27 NS
D (n = 20) 100 mg/kg NaMSA % p	 57.05 ± 10.23 93.22 NS	 76.29 ± 33.49 38.13 ≤ 0.010	 74.87 ± 8.29 28.54 ≤ 0.005

n – number of animals.
p – in comparison with the control group.

n – liczba zwierząt.
p – w porównaniu z grupą kontrolną.

Table 5. Renal mercury concentrations in groups K, A, B, C, D

Tabela 5. Stężenie rtęci w tkance nerkowej w grupach K, A, B, C, D

Group (Grupa)	Mean renal mercury concentration (Średnie stężenie rtęci w nerkach) mg/100 g of tissue
K (n = 20) %	5.72 ± 1.48 100
A (n = 20) 50 mg/kg NaQSA % p	2.83 ± 0.69 49.56 ≤ 0.001
B (n = 20) 100 mg/kg NaQSA % p	1.84 ± 0.47 32.25 ≤ 0.001
C (n = 20) 50 mg/kg NaMSA % p	4.82 ± 1.07 84.26 ≤ 0.05
D (n = 20) 100 mg/kg NaMSA % p	3.28 ± 0.99 57.34 ≤ 0.001

n – number of animals.
p – in comparison with the control group.

n – liczba zwierząt.
p – w porównaniu z grupą kontrolną.

while injury of the kidney is caused by selective mercury ion accumulation in this organ. The poisoning with inorganic mercury compounds has two phases. The first phase is characterized by gastroenterits leading to dehydration, and even hypovolemic shock, while the second phase beginning 1–3 days after poison ingestion involves acute renal failure [1–3].

All $HgCl_2$-treated rats exhibited macroscopic signs of gastroenteritis, however, animals' deaths were observed to occur mainly in the second post-poisoning week, what suggests that not gastroenteritis but renal failure was the cause of death. It is according to literature reports [1, 3, 12]. This conclusion is indirectly corroborated by the lowest mortality in rats having the lowest mercury concentration in the kidney (group B). The highest mortality, the largest mercury concentration in the renal tissue and the most voluminous damage in the microscopic picture of the kidney were seen in the control group. In contrast to the remaining groups, in the kidneys of control rats, necrosis affected not only proximal but also distal tubules and was accompanied by accumulation of calcium deposits in the tubule wall.

Application of NaQSA or NaMSA as antidotes proved efficient in groups A, B and D as it significantly lowered the mortality of the poisoned animals (table 1). Moreover, the results of biochemical tests and histopathological examination indicated in these groups the lowest kidney damage in comparison with the control group (tables 3–4). Only in group C, NaMSA treatment neither decreased mortality of the poisoned animals (table 1) nor did it protect them from renal damage which was indicated by a rise in urea and creatinine concentrations almost to the control level (Tables 3–4).

In all groups the treatment with NaQSA or NaMSA decreased also mercury absorption from the digestive tract and mercury accumulation in the kidneys (table 5). Despite lower renal mercury concentration in group C vs. control group, it was high enough to cause severe renal damage leading to the loss of 45 % of animals. Intragastrical NaQSA administration at a single dose of 50 or 100 mg/kg b.w. and single intragastrical NaMSA treatment at 100 mg/kg b.w. lowered the mortality of rats poisoned acutely with an inorganic mercury compound – $HgCl_2$. The mechanism of detoxifying action of NaQSA and NaMSA is most probably based on their ability to form insoluble complexes with mercury ions, which are not absorbed from the digestive tract and are excreted in feces.

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Address for correspondence:

Jan Magdalan
Department of Pharmacology Silesian Piasts University of Medicine in Wrocław
Mikulicza-Radeckiego 2
50-345 Wrocław
Poland
Tel.: +48 71 784 14 38
Fax: +48 71 784 00 94
E-mail: naladgam@op.pl

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