Quercetin-5’-sulfonic acid sodium salt and morin-5’-sulfonic acid sodium salt as antidotes in the subacute cadmium intoxication in mice

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Abstract:
The efficacy of quercetin-5’-sulfonic acid sodium salt (NaQSA) and morin-5’-sulfonic acid sodium salt (NaMSA) as antidotes in the subacute cadmium (Cd) intoxication was studied in mice. The administration of cadmium chloride (0.64 mg/kg/day, sc for 28 days) led to Cd accumulation in the liver and kidneys, induced lipid peroxide production in the liver, and decreased weight gain. Treatment with NaQSA and/or NaMSA diminished Cd accumulation in internal organs and Cd toxicity. NaQSA (20 mg/kg, ip for 28 days) exerted a stronger effect than NaMSA (20 mg/kg, ip for 28 days). Action of NaQSA and NaMSA used concomitantly (10 mg/kg, ip for 28 days) was not greater than the action of these substances used alone.

Key words: cadmium intoxication, NaQSA, NaMSA, LPO, mice


Introduction

Cadmium (Cd), a nonessential heavy metal, is a serious environmental and industrial pollutant [5]. Exposure to Cd induces oxidative stress in tissues of humans and experimental animals [2, 6]. Cd generates reactive oxygen species (ROS), which deplete endogenous ROS scavengers. ROS also damage a variety of transport proteins, including the Na⁺/K⁺-ATPase, which are subsequently degraded by the endolysosomal proteases. If these ROS-mediated stress damages are not balanced by repair processes, affected cells undergo apoptosis or necrosis [6, 20, 22]. Cd accumulates in tissues, especially in the kidney, which is one of the most sensitive organs [5, 16, 22]. Because of the very long half-life of cadmium in organs (15–20 years), toxic effects of this element could be observed for a long time after the exposure, often till the end of human’s life [20].
Until now, no substances lowering cadmium body burden and decreasing its toxic effects have been available. Chronic administration of known chelating compounds is not sufficiently effective and could be harmful [5]. There is a great necessity to find new, non-toxic but efficacious substances chelating Cd(II) ions.

In biological systems, flavonoids, a group of polyphenolic compounds ubiquitously found in vegetables, may act as antioxidants, with mechanisms involving both free radical-scavenging and metal chelation [13]. 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, like quercetin-5'-sulfonic acid sodium salt (NaQSA) and morin-5'-sulfonic acid sodium salt (NaMSA), characterized by good aqueous solubility, form complexes with Cd(II) and exhibit strong antioxidant activity [1, 9–12, 14, 15, 17]. It is worth emphasizing that NaQSA and NaMSA exert low toxicity. Nowadays, a few studies on new features of these compounds, eg. hypolipemic and antiatherogenic were conducted [7]. Therefore, they are supposed to be useful for a long-term therapy in patients chronically exposed to cadmium.

The aim of the present study was to assess the efficacy of NaQSA and NaMSA as antidotes (antioxidants) in the subacute cadmium intoxication in mice.

Materials and Methods

Animals

The study was conducted on 90 Balb/c male mice with body weight averaging 19.69 ± 1.6 g. Animals were housed up to five per cage, at 22°C and 40% humidity under 12 h light-dark cycle, with free access to water and standard granulated food (LSM, “Agropol”, Motycz).

Chemicals

The oxidative stress in mice was elicited by the administration of cadmium chloride anhydrous (Fluka Chemie, Switzerland). Cadmium chloride anhydrous (CdCl₂) was chosen because of its excellent solubility in water (140 g/100 g) [23]. CdCl₂ was administered subcutaneously (sc) at 0.64 mg/kg, which corresponds to 1/20 of their subcutaneous LD₅₀ in mice [19].

The sulfonic quercetin derivative NaQSA and the sulfonic morin derivative NaMSA were used as antioxidants. Both substances were applied intraperitoneally (ip) at doses of 10 or 20 mg/kg, which correspond to 1/80 and 1/40 of their intraperitoneal LD₅₀ in mice. NaQSA and NaMSA are characterized by good solubility in water and low toxicity to laboratory animals (mice and rats) [12, 21].

NaQSA and NaMSA were synthesized in the Department of Inorganic and Analytical Chemistry, University of Technology in Rzeszów, Poland, according to the methods described previously [10]. The purity of the obtained compounds was checked with thin-layer chromatography on alumina plates covered with an adsorbent (silica gel 60 WF₂₅₄, 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, the number of crystalline water molecules was determined 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 [9].

NaQSA and NaMSA 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 5.010⁻³ mol/dm³, while the aqueous solubility of NaMSA under the same conditions was 2.7 × 10⁻² mol/dm³. Sulfonic quercetin and morin derivatives can be considered to be multiprotonic acids, which dissociate in aqueous solutions yielding respective anions. Their dissociation constants (pKₐ) in aqueous solution determined at 20°C and I = 0.1 by potentiometric method were: pKₐ₁ = 4.67; pKₐ₂ = 7.84; pKₐ₃ = 9.82; pKₐ₄ = 10.69 for NaMSA, and pKₐ₁ = 7.43; pKₐ₂ = 8.16; pKₐ₃ = 9.24; pKₐ₄ = 10.84 for NaQSA [9, 10].

Experimental procedure

Mice were randomized to five control groups: K0 – mice receiving only 0.9% saline solution, K1 – mice...
receiving NaQSA, K2 – mice receiving NaMSA, K3 – mice receiving both NaQSA and NaMSA and K4 – mice receiving CdCl2, and three experimental groups: A – mice treated with both CdCl2 and NaQSA, B – mice treated with both CdCl2 and NaMSA and C – mice treated with CdCl2 and both flavonoids. CdCl2 was administered at a dose of 0.64 mg/kg, NaQSA and NaMSA were given at a dose of 20 mg/kg in groups: K1, K2, A and B or 10 mg/kg in groups K3 and C. Each control group comprised 12 animals and each experimental group comprised 10 animals. Saline solution, cadmium and studied substances were administered once a day, for 28 consecutive days. Cadmium, NaMSA and NaQSA were dissolved in 0.9% saline solution. CdCl2 was given sc in a volume of 5 ml/kg. Saline solution and flavonoids were given ip, in a volume of 10 ml/kg. The different routes of administration of CdCl2 and studied flavonoids were chosen to avoid complex formation.

At the end of the experiment the kidneys and livers were removed, washed and perfused with heparinized ice-cold normal saline solution. The livers were cut into two pieces. Kidneys and half of liver pieces were mineralized, then the cadmium content was assayed by atomic absorption spectrometry (SOLAAR M6 Spectrometer, ThermoElemental) and expressed in µg/100 g of wet tissue. Another half of liver pieces were homogenized on ice, using lysis buffer (140 mM NaCl, 10 mM EDTA, 10% glycerol, 1% NP40, 20 mM Tris base, pH 7.5). The homogenized tissues were centrifuged thereafter at 4°C with 14,000 rpm during 25 min and supernatants were taken [16]. Lipid peroxides (LPO) in the liver homogenates were measured as malondialdehyde production in the thiobarbituric acid reaction, using the colorimetric method and were expressed in µmol/l [18].

The experiment was performed after approval by the Local Ethics Commission for Experiments on Animals in Wroclaw (license no. 59/2006).

### Statistical analysis

Data were expressed as the mean values ± SD. Statistical analysis of the effect of factors on body weight, LPO and cadmium concentrations were performed using multifactor analysis of variance (ANOVA). Specific comparisons were made with contrast analysis. The STATISTICA 6.0 software was used, p < 0.05 was considered to be statistically significant.

### Results and Discussion

In group of mice exposed to Cd (group K4), significantly higher concentration of this element in the liver and kidneys and intensified lipid peroxidation in the liver compared to the group of mice receiving only saline solution (group K0) was observed (p ≤ 0.001 in both cases) (Fig. 1, 2, 3). Flavonoids administration (groups A, B and C) prevented the increase in Cd concentration in the liver and kidneys and the differences in comparison to group K4 were statistically significant (p ≤ 0.001 in all cases). NaQSA exerted a stronger effect on Cd accumulation in internal organs than NaMSA; significant differences between group A and B (p = 0.006) and group A and C (p ≤ 0.001) were revealed. In an earlier study, quercetin exerted a protective effect on cisplatin-induced nephrotoxicity in rats, which was results from its antioxidative features [3]. Data obtained from in vitro studies showed that quercetin-5’-sulfonic acid most effectively formed complexes with Cd at pH = 1.3–1.7 [11], which is more acidic environment than plasma. However, we could suspect that flavonoid-Cd complexes were formed after NaQSA and/or NaMSA administration and these complexes were probably excreted with urine but not accumulated in organs, because Cd concentration in the liver and kidneys was significantly lower than in the group of exposed, non-treated mice. Sulfonic derivatives of quercetin and morin are acidic compounds, therefore, it could be suspected that flavonoid-Cd complexes were rapidly excreted with the urine similarly to complexes of mercury with 2,3-dimercaptopropane-1-sulfonate (Unithiol) – a sulfonic derivative of 2,3-dimercaptopropanol (BAL) [5]. Therefore, the mechanism of formation and pharmacokinetics of flavonoid-Cd complexes requires further studies.

Flavonoids administration (groups A, B and C) prevented also the increase in LPO concentration in the liver. The differences in comparison with group K4 were statistically significant, (p ≤ 0.001 in all cases). These results revealed antioxidant properties of NaQSA and NaMSA. Mechanism of such effect could be related not only to the flavonoid-Cd complex formation but also to inhibition of oxygen free radical action by their scavenging [1, 13, 15]. There was no difference in the antioxidant activity between NaQSA and NaMSA. However, another study reported that quercetin, as a native compound, revealed a much
NaQSA and NaMSA as antidotes in subacute Cd intoxication

Fig. 1. Influence of subacute cadmium intoxication and flavonoids (NaMSA and NaQSA) administration on cadmium concentration in the liver.

Fig. 2. Influence of subacute cadmium intoxication and flavonoids (NaMSA and NaQSA) administration on cadmium concentration in the kidneys.
Fig. 3. Influence of subacute cadmium intoxication and flavonoids (NaMSA and NaQSA) administration on LPO concentration in the liver

0 – without NaMSA  1 – with NaMSA

Fig. 4. Influence of subacute cadmium intoxication and flavonoids (NaMSA and NaQSA) administration on body weight changes

0 – without NaMSA  1 – with NaMSA
stronger antioxidative action than morin. Under specific conditions, the latter substance might exert even a prooxidative effect [14].

Effects of NaQSA and NaMSA used concomitantly on Cd concentration in the liver and kidneys and LPO concentration in the liver were not greater than the action of these substances used alone.

Only in the group of animals exposed to Cd (without antidote – group K4), the increase in body weight was significantly inhibited in comparison with group K0 (p = 0.03) (Fig. 4). The design of this experiment allowed to exclude the decrease in food intake as a reason of body weight loss. Other studies also described loss of body weight in animals exposed to Cd. The mechanism by which Cd reduces body weight has been ascribed to a direct toxic action of Cd in the tissues. It has been shown that exposure to Cd leads to alterations in the hormonal metabolism and reduces the absorption of essential metals to the organism, which may result in anemia and weight loss in exposed individuals [4, 8].

No influence of NaQSA or/and NaMSA on body weight increase during 28 days of the experiment was observed (K1, K2, K3 vs. K0, p = NS). The body weight gain in mice subacutely intoxicated with Cd was significantly reduced (K4 vs. K0, p = 0.03). NaQSA and/or NaMSA administration (groups A, B and C) prevented the cadmium-induced body weight changes (A, B, C vs. K0, p = NS).

In our study, the antagonistic effect of NaQSA or/and NaMSA on cadmium toxicity was revealed. Protective effect of NaQSA and NaMSA against Cd prooxidative action could be probably the result of both: direct potent antioxidative activity of the studied flavonoids and Cd chelation by these substances [1, 9–15]. However, only subacute model of cadmium intoxication of mice was examined in this experiment. Therefore, further studies upon usefulness of NaQSA and NaMSA in chronic cadmium exposure are required.

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