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**Influence of water-soluble flavonoids: quercetin-5'-sulfonic acid sodium salt and morin-5'-sulfonic acid sodium salt on antioxidant parameters in the subacute cadmium intoxication mouse model**

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**Abstract**

Water-soluble quercetin-5'-sulfonic acid sodium salt (NaQSA) and morin-5'-sulfonic acid sodium salt (NaMSA) could exert an antagonistic effect on cadmium intoxication. The aim of the study was to examine the influence of these substances on superoxide dismutase (SOD) and glutathione (GSH) levels in the mouse liver in the subacute cadmium intoxication model. NaQSA and NaMSA significantly counteracted cadmium-induced decreases in SOD and GSH levels. No significant differences in SOD and GSH levels between groups exposed to cadmium receiving NaQSA or/and NaMSA were observed.

**Key words:** cadmium intoxication, NaQSA, NaMSA, morin, quercetin, flavonoids, GSH, SOD, antioxidants

**Introduction**

Cadmium (Cd) is a widespread environmental xenobiotic. One of the mechanisms of harmful effects of cadmium exposure is indirect induction of oxidative stress (Noor et al., 2002). Cadmium decreases superoxide dismutase (SOD), catalase, and glutathione peroxidase activity and inhibits antioxidant protein activity *via* binding with glutathione (GSH) thiol groups (Eybl et al., 2004; Stohs and Bagchi, 1995).

Despite of availability of chelating agents used in lowering cadmium body burden, there is a great necessity to find new, non-toxic but efficacious substances decreasing cadmium toxicity. *In vitro* studies have indicated that some compounds belonging to polyhydroxyflavones, e.g. morin and quercetin form complexes with Cd (II) and exhibit strong antioxidant activity. Their sulfonic water-soluble derivatives exert low toxicity therefore could be potential antidotes (Kopacz and Kuźniar, 2003; Magdalan et al., 2006a; Magdalan et al., 2006b; Szeląg et al., 2003).

In our previous study we have demonstrated the antagonistic effect of quercetin-5'-sulfonic acid sodium salt (NaQSA) and morin-5'-sulfonic acid sodium salt (NaMSA) on Cd-induced hepatic lipid peroxidation in the subacute Cd intoxication in mice (Magdalan et al., 2007).

The aim of the study was to examine the influence of NaQSA and NaMSA on SOD activity and GSH concentration in the mouse liver in the subacute cadmium intoxication model.

### Materials and Methods

The study was conducted in 90 Balb/c male mice ( $19.7 \pm 1.6$  g), housed at standard laboratory conditions with free access to water and food.

In the experiment were used: cadmium chloride anhydrous ( $\text{CdCl}_2$ ) (Fluka Chemie, Switzerland), NaQSA and NaMSA synthesized in the Department of Inorganic and Analytical Chemistry, University of Technology in Rzeszow, Poland. NaQSA and NaMSA are water soluble substances of which characteristics were described in earlier papers (Kopacz and Kuźniar, 2003; Magdalan et al., 2006a; Magdalan et al., 2006b; Szeląg et al., 2003).

Mice were randomized to 8 experimental groups ( $n=10-12$ ) receiving: K – only 0.9 % saline solution, Q – NaQSA, M – NaMSA, QM – NaQSA and NaMSA, Cd – receiving  $\text{CdCl}_2$ , CdQ –  $\text{CdCl}_2$  and NaQSA, CdM –  $\text{CdCl}_2$  and NaMSA and group CdQM –  $\text{CdCl}_2$  and both flavonoids, respectively.

Cadmium chloride was administered subcutaneously (*sc*) at a dose of 0.64 mg/kg b.w. NaQSA and NaMSA were given intraperitoneally (*ip*) at a dose of 20 mg/kg b.w. in groups: Q, M, CdQ and CdM or 10 mg/kg b.w. in groups QM and CdQM. All substances were dissolved in 0.9% saline solution and administered once a day, for 28 consecutive days. The different routes of administration of  $\text{CdCl}_2$  and the studied flavonoids were chosen to avoid complex formation.

Livers were homogenized on ice, using lysis buffer, centrifuged with 14000 rpm during 25 min. In the obtained supernatants SOD activity and GSH concentration were measured.

SOD activity in liver homogenates was assayed spectrophotometrically using RANSOD kit (Randox Laboratories, UK) according to the manufacturer's instructions. SOD activity was expressed as U/mg protein. Total protein concentrations were determined by the Total Protein Kit (Sigma).

Glutathione concentration in liver homogenates was measured using colorimetric assay BIOXYTECH GSH-400 (OxisResearch, USA) according to the manufacturer's instructions. GSH level was expressed as  $\mu\text{M/L}$ .

The experiment was performed after approval by the Local Ethics Commission for Experiments on Animals in Wrocław.

Data were expressed as the mean values  $\pm\text{SD}$  (standard deviation). Statistical analysis was performed using multifactor analysis of variance (ANOVA). Specific comparisons were made with contrast analysis, p value  $<0.05$  was considered to be statistically significant.

## Results

In the group receiving only cadmium GSH level and SOD activity in the mouse liver were significantly lower than in groups non-exposed to this metal (group Cd vs. K, Q, M and QM;  $p \leq 0.001$  in all cases). No significant differences in GSH level and SOD activity between groups non-exposed to cadmium were revealed (groups K, Q, M and QM;  $p = \text{NS}$  in all cases). In groups receiving cadmium with one or both examined flavonoids, SOD activity and GSH level were significantly higher than in the group receiving only cadmium (CdQ, CdM and CdQM vs. Cd;  $p \leq 0.001$  in all cases). Concomitant flavonoids and cadmium administration significantly prevented the cadmium-induced decrease in GSH concentration and SOD activity, however, these changes were not fully reversible (groups CdQ, CdM and CdQM vs.

K, Q, M, and QM;  $p \leq 0.001$  in all cases). No significant differences in SOD activity and GSH concentration between groups exposed to cadmium receiving one or both of the examined flavonoids were observed (groups CdQ, CdM and CdQM;  $p = \text{NS}$  in all cases). GSH level and SOD activity were higher in group CdQ than in group CdM, however, all these differences were not statistically significant (Figure 1).

## Discussion

In our previous study we have demonstrated that in the subacute cadmium intoxication model in mice this metal significantly accumulated in the liver and kidneys and exerted Cd-induced prooxidative action. Intraperitoneal administration of NaQSA and NaMSA prevented lipid peroxidation in the liver and decreased Cd level in the organs (Magdalan et al., 2007). Therefore, more detailed studies on the mechanisms of antioxidative action of NaQSA and NaMSA in the same cadmium poisoning mouse model were conducted. Influence of both examined flavonoids on selected nonenzymatic (GSH) and enzymatic (SOD) antioxidative parameters was studied.

Animal studies have shown that flavonoids exhibit potent antioxidant activity with mechanisms involving free radical-scavenging, metal chelation, antioxidant enzyme activation, reduction alpha-tocopherol radicals and oxidase inhibition (Fang and Yang, 2002). Mechanism of protective action of NaQSA and NaMSA against Cd-induced prooxidative status could be probably a consequence of direct antioxidative influence and/or Cd chelation by the examined flavonoids. It was demonstrated that both flavonoids were effective antidotes in acute other metal poisoning also e.g. chromium and mercury (Kopacz and Kuźniar, 2003; Magdalan et al., 2006a; Magdalan et al., 2006b; Szeląg et al., 2003).

Superoxide dismutase is one of the most important antioxidative enzymes. SOD catalyzes the conversion of single electron reduced species of molecular oxygen to hydrogen peroxide and oxygen (Fang and Yang, 2002; Noor et al., 2002). The cadmium-induced

inhibition of many metalloenzymes is reported to be the result of the displacement of metals from the active site of the enzymes. It was observed that cadmium administration inhibited the activity of SOD *in vitro* and *in vivo* in many tissues e.g. erythrocytes, kidneys and liver (Stohs and Bagchi, 1995). In our study we have obtained similar results. We demonstrated that subacute cadmium intoxication significantly inhibited superoxide dismutase activity in the mouse liver.

Many data indicate protective properties of flavonoids, esp. quercetin against oxidative stress induced by different agents. It was found that quercetin successfully attenuated toxic effects of CCl<sub>4</sub> by influence on SOD activity in the rat liver (Amalia et al., 2007). Quercetin also countered the pro-oxidant effects of galactose-induced hyperglycemic oxidative stress in rats, significantly reversing changes in SOD activity (Ramana et al., 2006).

In rats, during chronic cadmium administration, renal superoxide dismutase activity was higher in the group receiving Cd with quercetin than in the group receiving only Cd (Morales et al., 2006). In our study we also observed similar action. In livers isolated from cadmium-intoxicated mice treated with NaQSA (group CdQ), significantly higher SOD activity was observed in mice receiving only cadmium (group Cd). Till now there was no report on the influence of quercetin or its water-soluble derivatives administered alone on SOD activity. It was observed that a single dose of morin significantly elevated SOD activity in normal mice. Pretreatment with this flavonoids also prevented whole body gamma irradiation-induced drastic decrease in endogenous SOD activity (Parihar et al., 2007). In our work we did not observed significant influence of NaQSA and/or NaMSA on the mouse liver SOD activity (groups M, Q and QM) (Figure 1).

Reduced glutathione, an extremely important cell protectant, is stored mainly in the liver. Glutathione keeps redox-sensitive active sites in enzymes in the reduced state (Wu et al., 2004). It was shown that cadmium produced an increase in intracellular glutathione

concentrations in a dose- and time-dependent manner. At higher Cd doses a decreased GSH level was observed probably as a result of generation of reactive oxygen species exceeding the ability to regenerate reduced glutathione (Stohs and Bagchi, 1995). In our work we observed significant glutathione depletion probably as a result of glutathione storage consumption after 28 days of cadmium poisoning (Figure 1).

Quercetin may influence GSH/GSSG (reduced glutathione/glutathione disulfide) ratio and protein thiolation (Meyers et al., 2008). Quercetin treatment produced an increase in the GSH/GSSG ratio in hepatic tissue but not in the plasma or cardiac tissue. Pretreatment with quercetin may protect against ethanol-induced oxidative stress indirectly by enhancing the production of the endogenous liver GSH (Molina et al., 2003). Quercetin administration successfully diminished toxic effects of CCl<sub>4</sub> by influence on GSH concentration in the rat liver (Amalia et al., 2007). Quercetin also countered the pro-oxidant effects of galactose-induced hyperglycemic oxidative stress significantly reversing changes in reduced GSH level (Ramana et al., 2006).

It was demonstrated that in chronic cadmium administration resulted in higher renal glutathione-reductase activity in the rats receiving Cd with quercetin than those receiving only cadmium (Morales et al., 2006). In our experiment we observed that NaQSA significantly prevented cadmium-induced GSH depletion in the mouse liver (Figure 1).

It was observed that intraperitoneal administration of morin (100 mg/kg) significantly increased not only SOD activity but also liver GSH level in normal mice. Pretreatment with this flavonoid also prevented whole body gamma irradiation-induced drastic decrease in endogenous GSH concentration (Parihar et al., 2007). Similarly to SOD activity in our work we did not observe significant influence of NaQSA and/or NaMSA on GSH liver amount during 4 weeks of the study (groups Q, M, and QM) (Figure 1). Lack of the influence of these

two compounds on SOD and GSH levels may be due to lower doses of NaQSA and NaMSA used in our experiment vs. earlier studies.

Our study revealed the significant difference between NaQSA and NaMSA efficacy nor the synergism of both examined flavonoids in the antioxidant action against cadmium toxicity, monitored by GSH levels and SOD activity in mice liver (Figure 1).

Our results may be different from those obtained in other experiments. In previously conducted studies natural, lipophilic quercetin or morin were used, whereas in our experiments water-soluble derivatives of these flavonoids were investigated. Different pharmacokinetic properties may play an important role in gastrointestinal absorption, distribution, metabolism or excretion of these compounds what subsequently could be the reason of various biological effects of hydrophilic and lipophilic compounds. It was shown that sulfonic derivatives of natural morin and quercetin were less potent than the original agents in their cytostatic and cytotoxic activities. However, their solubility in water was greater than that of the original agents and higher culture medium concentrations of NaMSA and NaQSA were obtained (Król et al., 2002).

In our study we revealed antioxidant activity of NaMSA or/and NaQSA. Both substances significantly counteracted Cd-induced decrease in SOD activity and GSH depletion in the mouse liver, however, Cd-induced changes were not fully reversible. It could not be excluded that higher doses of NaQSA and/or NaMSA may exert stronger effect. There was no difference in the antioxidant activity between NaQSA and NaMSA against cadmium poisoning in mice probably due to similar (may be even the same) mechanism of action. These findings seem to be very promising but required further studies.

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**Figure legends**

Figure 1. Influence of subacute cadmium intoxication and flavonoids (NaMSA and NaQSA) administration on SOD activity and GSH level in the mouse liver.

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