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Effect of Quercetin-5'-sulfonic Acid Sodium Salt on SOD Activity and ADMA/DDAH Pathway in Extracorporeal Liver Perfusion in Rats*

Wpływ soli sodowej kwasu kwercetyno-5'-sulfonowego na aktywność dysmutazy ponadtlenkowej i układ ADMA/DDAH podczas pozaustrojowej perfuzji wątroby u szczurów

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Abstract

Background. Quercetin-5'-sulfonic acid sodium salt (NaQSA) exerts good aqueous solubility, strong antioxidant activity and low toxicity.
Objectives. The aims of this study were to investigate the effect of NaQSA on superoxide dismutase (SOD) activity and ADMA/DDAH pathway during extracorporeal liver perfusion (ELP).
Material and Methods. The study was carried out on male Wistar rats. Isolated livers were perfused with Krebs-Henseleit bicarbonate buffer (KHB) + 1 μM ADMA (group C), or with KHB + 1 μM ADMA and either 10 μM NaQSA (Q10) or 50 μM NaQSA (Q50). In group 0 (sham) livers were perfused with KHB alone. Levels of ADMA, alanine (ALT) and aspartate (AST) aminotransferases activities were measured during perfusion. After 90 min. of perfusion superoxide dismutase (SOD) and dimethylarginine dimethylaminohydrolase (DDAH) activities were estimated in liver homogenates.
Results. DDAH activity in Q10 group was significantly higher as compared to control and Q50 groups. No significant differences were observed between Q50 and control group. The decrease in ADMA concentration during perfusion was observed in all groups, but the most pronounced in the group Q10 and the least in group Q50. During perfusion AST activities were the lowest in Q50 group. No significant difference in SOD activity in groups perfused with NaQSA as compared to control group was noted.
Conclusions. The impact of NaQSA on ADMA/DDAH system depends on its concentration. In lower concentration NaQSA exerted some beneficial properties which vanished in higher concentration. No increase in SOD activity during perfusion with NaQSA was observed (**Adv Clin Exp Med 2012, 21, 4, 423–431**).

Key words: quercetin, extracorporeal perfusion, oxidative stress, ADMA, DDAH, rats.

Streszczenie

Wprowadzenie. Sól sodową kwasu kwercetyno-5'-sulfonowego (NaQSA) cechuje dobra rozpuszczalność w wodzie, silne działanie antyoksydacyjne i mała toksyczność.
Cel pracy. Ocena wpływu NaQSA na aktywność dysmutazy ponadtlenkowej (SOD) i układ ADMA/DDAH podczas pozaustrojowej perfuzji wątroby (ELP).
Materiał i metody. Badanie przeprowadzono na samcach szczurów szczepu Wistar. Izolowane wątroby perfundowano buforem Krebsa-Henseleita (KHB) + 1 μM ADMA (grupa C), lub KHB + 1 μM ADMA z dodatkiem 10 μM

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NaQSA (grupa Q10) lub 50 μM NaQSA (grupa Q50). W grupie 0 (sham) wątroby perfundowano jedynie KHB. Podczas perfuzji zostały oznaczone poziom ADMA oraz aktywność aminotransferaz alaninowej (ALT) i asparaginianowej (AST). Po 90 min perfuzji w homogenatach wątrób oznaczono aktywności dysmutazy ponadtlenkowej (SOD) i dimetyloaminohydrolazy dimetyloargininy (DDAH).
Wyniki. Aktywność DDAH w grupie Q10 była istotnie większa w porównaniu z grupą kontrolną i Q50. Nie stwierdzono istotnych różnic aktywności DDAH między grupami Q50 i kontrolną. Zmniejszenie stężenia ADMA w czasie perfuzji obserwowano we wszystkich grupach, największe w grupie Q10, a najmniejsze w grupie Q50. Stężenie AST podczas perfuzji było najmniejsze w grupie Q50. Nie stwierdzono istotnych różnic w aktywności SOD w grupach perfundowanych NaQSA w porównaniu z grupą kontrolną.
Wnioski. Wpływ NaQSA na układ ADMA/DDAH zależy od jej stężenia. W mniejszym stężeniu NaQSA wykazywał korzystne właściwości, które zniknęły w większym stężeniu. Nie obserwowano wzrostu aktywności SOD podczas perfuzji z NaQSA (**Adv Clin Exp Med 2012, 21, 4, 423–431**).

Słowa kluczowe: kwercetyna, perfuzja pozaustrojowa, stres oksydacyjny, ADMA, DDAH, szczury.

Nitric oxide (NO), an endothelium-derived relaxing factor, inhibits expression of many chemoattractants, platelet aggregation and adhesion of leukocytes, decreases oxidation of low-density lipoprotein (LDL), and suppresses abnormal proliferation of vascular smooth muscle cells. In the liver, NO is responsible for maintaining sinusoidal tone and flow in liver sinusoidal vessels [1]. It also evokes relaxation of hepatic stellate cells [1, 2]. NO is synthesized constitutively by endothelial nitric oxide synthase (eNOS) which is responsible for basal production of NO [3, 4]. Decreased level of this compound may promote progression of vascular diseases [5]. On the other hand, excessive amounts of NO produced by inducible nitric oxide synthase (iNOS) are involved in the inflammatory process and organ injury [6, 7].

Asymmetric dimethylarginine (N^{G},N^{G}-dimethyl-L-arginine, ADMA), one of the methylated amino acids derived from arginine, inhibits all isoforms of NO synthase [8]. Oxidative stress is responsible for increased synthesis and/or inhibited metabolism of this compound [9]. Elevated plasma ADMA levels are associated with impaired endothelium-dependent vasodilatation [10] and in such pathological states as hypercholesterolemia, hyperglycaemia, hyperhomocysteinemia, hypertension, coronary artery disease, heart failure, and stroke, plasma levels of ADMA may be increased two- or even tenfold, contributing to inhibition of NO synthesis and endothelial dysfunction [10–14]. Impaired liver function may also lead to increased plasma levels of ADMA [15]. An elevated level of ADMA may be explained by decreased activity of dimethylarginine dimethylaminohydrolase (DDAH) [16], an enzyme located mainly in the liver and responsible for the metabolism of ADMA to citrulline and dimethylamine [16, 17].

Quercetin (5,7,3’,4’-pentahydroxyflavone) is one of the most popular flavonoids found in fruits and vegetables with many beneficial properties: antitumor, antithrombotic and antiviral activity. It exerts also vasodilatory effect, inhibitis cell proliferation, suppresses LDL-induced oxidation, stabilizes immune cells, and modulates eicosanoid production [18–20]. Quercetin was also demonstrated to reduce liver injury [21–23] and exert antioxidant activity, with mechanisms involving both free radical-scavenging and metal chelation [22, 24]. However, the influence of quercetin on NO production is not fully understood. Several studies show no or even negative action of this compound but others demonstrated opposite results [25, 26]. Antiatherogenic effect of quercetin may be due to its influence on ADMA metabolism.

The present study was designed to investigate the ability of NaQSA to prevent liver injury during extracorporeal liver perfusion (ELP), the effect of this compound on superoxide dismutase (SOD) activity and ADMA/DDAH pathway. Because bioavailability of natural quercetin is rather poor [27] the authors decided to use quercetin-5’-sulfonic acid sodium salt (NaQSA) which is characterized by good aqueous solubility and strong antioxidant activity [28–30]. It is worth emphasizing that NaQSA also exerts low toxicity [31].

Material and Methods

Animals

The study was carried out on adult male Wistar rats (504.6 ± 80.8 g) obtained from the Animal Laboratory of the Department of Pathological Anatomy, Wroclaw Medical University. The animals were housed individually in chambers with a 12:12 h light-dark cycle, temperature maintained at 21–23°C. Before the experiment animals had free access to standard food and water. The experiment was performed with the consent of the I Local Ethics Commission for Experiments on Animals in Wroclaw.

In this experiment 4 groups were arranged: group C (n = 9) – livers perfused with Krebs-Henseleit bicarbonate buffer (KHB) + 1 μM ADMA,

group Q10 (n = 8) – livers perfused with KHB containing 1μM ADMA and 10 μM NaQSA, group Q50 (n = 8) – livers perfused with KHB containing 1 μM ADMA and 50 μM NaQSA, group 0 (n = 7) – livers perfused with KHB alone. Group 0 (sham) was arranged to check the secretion of endogenous ADMA from liver during ELP.

Substances

NaQSA was synthesized in the Department of Inorganic and Analytical Chemistry, University of Technology in Rzeszow, Poland, according to the methods described previously [30]. The purity of the obtained compound was checked with thin-layer chromatography. 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 [32].

NaQSA is easily soluble in water and keeps properties of the parent compounds. The aqueous solubility of NaQSA at 22°C1°C (295 K) was estimated at 5.0×10^{-3} mol/dm^3. Sulfonic quercetin derivative can be considered to be multiprotonic acid, which dissociates in aqueous solutions with constant (pK_a) determined at 20°C and I = 0.1 by potentiometric method as follow: $pK_{a1} = 7.43$; $pK_{a2} = 8.16$; $pK_{a3} = 9.24$; $pK_{a4} = 10.84$ [30, 32].

ADMA (Sigma, Germany), heparin-amp. 25000U (Biochemie, Austria), 0.9% sodium chloride solution (Polpharma S.A., Poland), Ringer solution (Polfa Lublin S.A., Poland), thiopental – amp. 0.5 g (Biochemie, Austria), a modified Krebs-Henseleit bicarbonate buffer (KHB) (118 mM NaCl (Chempur, Poland), 25mM $NaHCO_3$ (Chempur, Poland), 4.8 mM KCl (Chempur, Poland), 1.5 mM $CaCl_2$ (Chempur, Poland), 1.2 mM $MgSO_4 \times 7H_2O$ (Polskie Odczynniki Chemiczne S.A., Poland), 1.2 mM KH_2PO_4 (Chempur, Poland), 4.9955 mM glucose (Fluka Chemie, Switzerland)), pH 7.4) were also used in this study.

Liver Isolation and Storage

The animals were anesthetized by intraperitoneal injection of thiopental at a dose of 70 mg/kg of body weight. Middle incision was made to open the abdominal cavity, inferior caval vein was ligated above the right renal vein opening, and a canule was introduced into the portal vein through which Ringer solution supplemented with heparin was perfused. Then, the chest was opened and the second canula was inserted into the inferior caval vein, pointing towards the liver. Then livers were excised and transferred as a whole to the chamber for ELP of this organ.

Extracorporeal Perfusion

Thereafter, the livers were placed in an anatomical position. Perfusion fluid, which was heated to 37°C and oxygenated with a mixture of 95% O_2 and 5% CO_2, flew into the liver through the canula inserted to the portal vein and left the organ through the canula introduced to the inferior caval vein. The perfusion fluid (KHB) was propelled by peristaltic pump at a flow rate of 20 ml/min. for the first 10 min. of perfusion and at 30 ml/min. thereafter. Pressure was monitored throughout the entire perfusion period and kept in the range between 4–5 mm Hg. The livers were perfused using Universal Perfusion System UNIPER UP – 100 (Hugo Sachs Elektronik, Harvard Apparatus GmbH) in an open-circuit mode.

Samples of perfusion fluid (1 mL) to assay ADMA levels were collected after 15, 45, 90 min and to assay alanine (ALT) and aspartate (AST) aminotransferases activities – after 15, 45, 60 and 90 min. of perfusion.

After 90 min the perfusion was terminated and livers were weighted and 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 thereafter centrifuged at 4°C with 14000 rpm during 25 min and supernatants were taken [33]. In the obtained supernatants SOD and DDAH activities were assayed.

Blood Enzymes Analysis

ADMA concentration was measured simultaneously by high-performance liquid chromatography (HPLC) with fluorescence detection [34, 35]. The plasma samples and standards were extracted on a solid-phase extraction cartridge with SCX 50 columns (Varian). The analytes were derivatized with o-phthaldialdehyde and separated by isocratic reversed-phase chromatography on a Symmetry C18 column (150 × 4.6 mm, 5-μm particle size; Waters Corp., Milford, MA, USA). Potassium phosphate buffer (50 mM, pH 6.6) containing 12% v/v acetonitrile was used as the mobile phase at a flow rate of 1.1 mL/min and a column temperature of 35°C. Fluorescence detection was performed at the excitation and emission wavelengths of 340 and 450 nm, respectively.

The serum activities of ALT and AST were assayed with a commercial enzymatic method (Biomerieux) in a certified laboratory. Activities of these enzymes and ADMA levels were expressed per gram of liver.

Tissue Enzymes Analysis

DDAH activity was measured spectrophotometrically according to the method of Tain and Baylis [36], adopted to the macromethod for spectrophotometer MARCEL S350 PRO. The method is based on the rate of L-citrulline production. Briefly, liver tissue homogenate were mixed with phosphate buffer, pH = 6.5. 1 mM ADMA was added to the samples which were then incubated in 37°C for 45 minutes. After the reaction was stopped with 4% sulfosalicylic acid, samples were centrifuged and oxime reagent (diacetyl monoxime (0.08% wt/vol) in 5% acetic acid) mixed with antipyrine/H_2SO_4 (antipyrine (0.5% wt/vol) in 50% sulfuric acid) reagent was added. Samples were thereafter incubated in 60°C for 110 minutes and cooled in ice bath for 10 minutes. L-citrulline formation was measured at 466 nm and values were subtracted of respective blanks (without ADMA). Standard was prepared as serial dilutions of L-citrulline. DDAH activity was represented as µM L-citrulline formation/g protein/min at 37°C.

SOD activity was estimated using RANSOD kit (Randox Laboratories, Crumlin, UK), according to the manufacturer's instructions.

Activity of SOD and L-citruline formation were recalculated for total protein content in supernatants. The concentration of protein was assayed with a commercial enzymatic method in a certified laboratory.

Statistical Analysis

Data was expressed as the mean values ± SD. Statistical analysis of the effect of examined compound on SOD and DDAH activities were performed using two-way analysis of variance (ANOVA). The impact of NaQSA and the time of perfusion on ALT, AST, and ADMA during perfusion were analyzed using MANOVA with repeats. Specific comparisons were made with contrast analysis. Hypotheses were considered positively verified if $p \le 0.05$. Statistica 8.0 software was used.

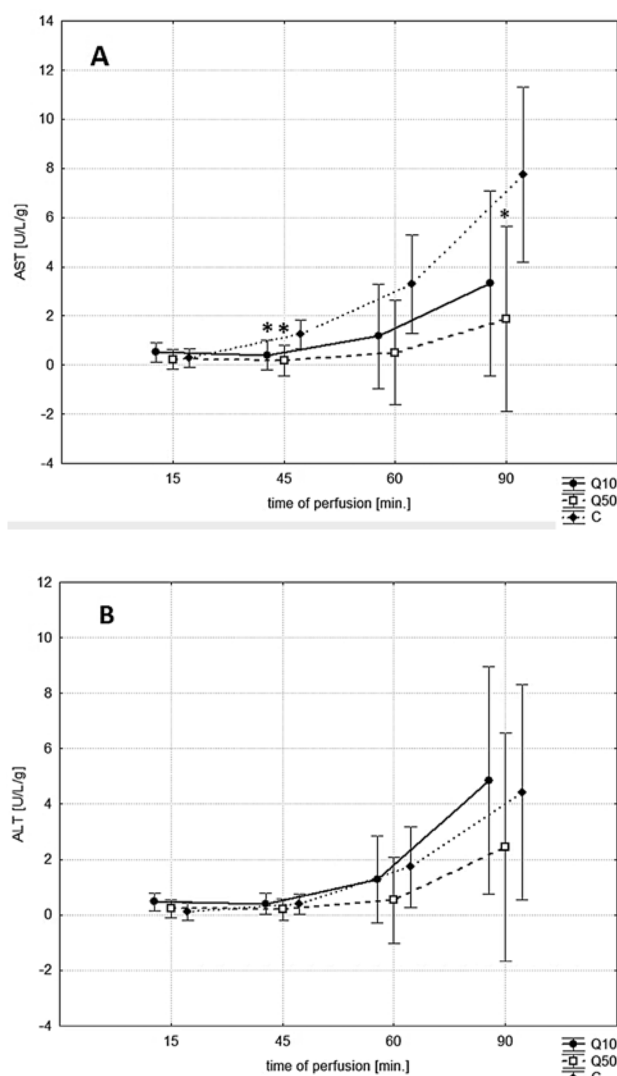

Fig. 1. Difference in average activity of AST [U/l] (Fig. 1A) and ALT [U/l] (Fig. 1B) per 1 gram of liver as a function of time of perfusion in three groups of livers: C, Q10, Q50. A significant influence of duration of perfusion on ALT and AST activities ($p < 0.001$ in both cases) was observed. In particular points of time significant differences between examined groups were indicated with asterisks. Detailed comparisons are presented in the Results section. Data was presented as mean ± SD

Ryc. 1. Średnie wartości aktywności AST [U/l] (ryc. 1A) i ALT [U/l] (ryc. 1B) na 1 gram tkanki wątroby w czasie perfuzji w 3 grupach: C, Q10, Q50. Stwierdzono istotny wpływ czasu trwania perfuzji na aktywność ALT and AST ($p < 0.001$ dla obu enzymów). W poszczególnych punktach czasowych różnice istotne statystycznie są zaznaczone za pomocą gwiazdek. Porównanie danych przedstawiono w sekcji Wyniki. Dane przedstawiono jako wartości średnie ± odchylenie standardowe SD

Results

AST and ALT

The study showed significant influence of perfusion duration on the activities of ALT and AST ($p < 0.001$ for both comparisons) (Fig. 1).

AST activities were lowest in group Q50 in 45th and 90th minutes of the perfusion. In the 45th minute of the perfusion AST activities were 0.186 ± 0.17 U/L/g in group Q50, and 0.402 ± 0.88 U/L/g in group Q10 and were significantly lower than in group C in which AST activity was 1.258 ± 1.1 U/L/g ($p < 0.05$ in both cases). In

the 90th minute of perfusion AST activity in group Q50 was 1.87 ± 2.97 U/L/g and was also significantly lower than in group C in which the value was 7.73 ± 6.4 U/L/g ($p < 0.05$) (Fig. 1A). In the 45th minute of perfusion in group Q50 ALT activity was 0.2 ± 0.2 U/L/g and was lower than in group Q10 (0.4 ± 0.81 U/L/g) and C (0.39 ± 0.37 U/L/g). However, no statistically significant differences in ALT activities were observed between groups at any time point (Fig. 1B).

In contrast to the group C, no increase in ALT and AST activities between 15th and 45th minute of perfusion was observed in groups Q10 and Q50. Therefore, significant differences in AST and ALT activities were observed in groups Q10 and Q50 compared to the group C (for AST $p < 0.005$ for both groups, and for ALT $p < 0.05$ for both groups). Increase in AST activity between 15th and 90th minute of the perfusion was significantly lower in group Q50 than in group C ($p < 0.05$) (Fig. 1).

SOD

No significant differences in SOD activity expressed per gram of protein in both groups perfused with NaQSA as compared to control group was noted. In Q50 group SOD activity was 1.61 ± 0.12 U/g and was significantly different ($p < 0.05$) from the values obtained in group Q10 in which the SOD activity was 1.42 ± 0.11 U/g (Fig. 2).

DDAH

DDAH activity in group Q10 was 0.99 ± ± 0.21 μM L-citrulline/g protein/min and was significantly higher as compared to control and Q50 groups ($p < 0.05$). No significant difference was observed between group C and Q50 ($p < 0.05$) (Fig. 2).

ADMA

The study showed significant influence of duration of perfusion on ADMA levels ($p < 0.001$) (Fig. 3). In the 15th and 45th minute of perfusion significant difference between group Q10 and group C was observed ($p < 0.05$ in both time points). ADMA value in group Q10 was the highest and reached 0.09 ± 0.025 μM/g in 15th minute and 0.08 ± 0.025 μM/g in the 45th minute of perfusion. At the same time points ADMA values in the group C were 0.065 ± 0.007 μM/g and 0.062 ± 0.004 μM/g, respectively.

Between the 15th and 45th minute of perfusion only in the Q50 group an increase in ADMA level was observed in opposite to observed decrease in ADMA levels in groups Q10 and C ($p < 0.05$ in both cases).

Between the 15th and 90th minute of perfusion a decrease in ADMA concentration was observed in all groups, the most pronounced in group Q10 and the least in group Q50. The difference between these two groups was, however, not significant ($p = 0.06$). In the 90th minute of the perfusion, ADMA level was highest in the group Q50 (0.067 ± 0.03 μM/g); however, the difference was not significant as compared to other groups.

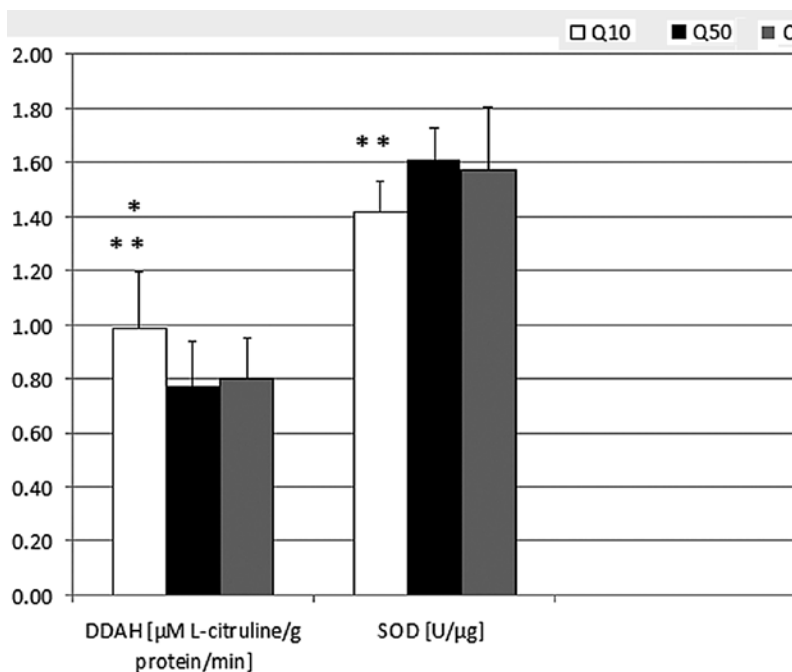

Fig. 2. Influence of NaQSA on DDAH and SOD activities after 90 minutes of perfusion in three examined groups of livers: C, Q10, Q50. Significant differences between examined groups were indicated with asterisks: in group Q10 DDAH activity was significantly higher ($p < 0.05$) than in group C (*) and in group Q50 (**). In Q10 group SOD activity was significantly lower ($p < 0.05$) than in the group Q50 (**). Detailed comparisons are presented in the Results section. Data was presented as mean ± SD

Ryc. 2. Wpływ NaQSA na aktywność DDAH i SOD po 90 minutach perfuzji w 3 badanych grupach: C, Q10, Q50. Aktywność DDAH w grupie Q10 była istotnie większa (p < 0,05) w porównaniu z grupą C (*) i grupą Q50 (**). Istotne statystycznie różnice przedstawiono za pomocą gwiazdek: aktywność SOD w grupie Q10 była istotnie mniejsza (p < 0,05) w porównaniu z grupą Q50 (**). Porównanie danych przedstawiono w sekcji Wyniki. Dane przedstawiono jako wartości średnie ± odchylenie standardowe SD

Table 1. Influence of time of perfusion on ADMA [µM] per 1 gram of rat liver in sham group (group 0, n = 7). ADMA values were on the border of sensitivity of the analytical method and no significant differences were observed between groups in any time points

Tabela 1. Wpływ czasu perfuzji na stężenie ADMA [µM] per 1 gram wątroby szczurów w grupie sham (grupa 0, n = 7). Wartości ADMA były na granicy czułości metody analitycznej i nie stwierdzono istotnych różnic między grupami w żadnym punkcie czasowym

Group 0 (sham) (Grupa 0 (sham)) [n = 7]		Time of perfusion (Czas perfuzji) – min		
		15	45	90
ADMA [µmol/L/g of liver]	mean	0.006	0.008	0.006
	±SD	0.002	0.002	0.002

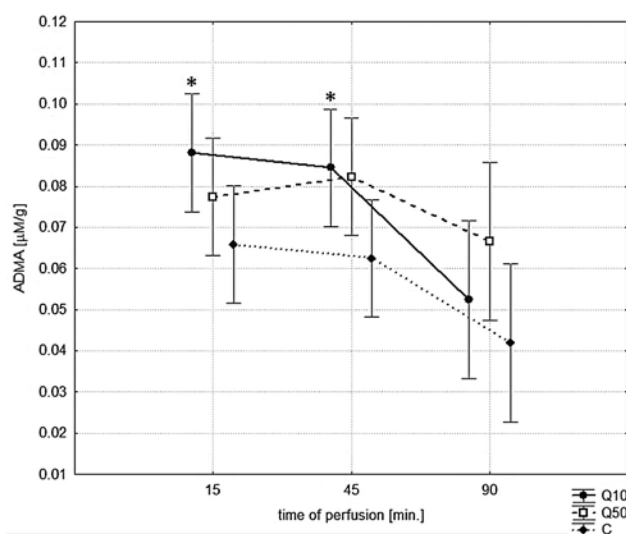

Fig. 3. The influence of NaQSA and the time of perfusion on ADMA level [µM] per 1 gram of rat liver in three examined groups of livers: C, Q10, Q50. A significant influence of duration of perfusion on ADMA levels ($p < 0.001$) was noticed. In particular points of time significant differences between examined groups were indicated with asterisks. Detailed comparisons are presented in the Results section. Data was presented as mean ± SD

Ryc. 3. Wpływ NaQSA i czasu trwania perfuzji na stężenie ADMA [µM]/gram wątroby szczurów w 3 badanych grupach: C, Q10, Q50. W poszczególnych punktach czasowych różnice istotne statystycznie są zaznaczone za pomocą gwiazdek. Porównanie danych przedstawiono w sekcji Wyniki. Dane przedstawiono jako wartości średnie ± odchylenie standardowe SD

In group 0 (sham) ADMA values were on the border of sensitivity of the analytical method and no significant differences were observed between groups in any time points (Table 1).

Discussion

In some experiments quercetin caused an increase in NO production [26, 37], enhanced eNOS activity [38, 39], can generate hydrogen peroxide [40, 41] or inhibit iNOS [6, 7] and neuronal nitric oxide synthase (nNOS) activity [42]. In present work the authors investigated the effect of NaQSA – characterized by good aqueous solubility, strong antioxidant activity and low toxicity [28–31] on ADMA/DDAH pathway. During the experiment the decrease in ADMA, NO inhibitor level, was observed in all groups in which ADMA was added to the perfusate. It seems that the increased DDAH activity, the enzyme responsible for ADMA metabolism, may be the reason of the decrease in ADMA level. In the group perfused with NaQSA at 50 µM the lowest decrease of ADMA level was observed. Moreover, between the 15th and 45th minute of the perfusion even the increase in ADMA level was noticed in this group, which was accompanied by the lowest activity of DDAH in liver homogenates after the perfusion. The highest decrease in ADMA level during the perfusion was observed in the group perfused with NaQSA at the concentration of 10 µM. In this group DDAH activity after the perfusion was the highest. In described experiment perfusion of liver based on an open-circuit mode and livers were perfused with pefusate supplemented with ADMA at the concentration of 1 µM. Therefore, we may assume that changes in ADMA values between subsequent time points reflected changes in liver function during those periods of perfusion. It is worth emphasizing that the impact of endogenously produced ADMA is negligible because ADMA levels in the group of livers perfused with KHB only (sham) were minimal (near the limit of the method's sensitivity) and they remained constant throughout the perfusion and probably did not significantly change the experiment outcome.

Such results may also suggest that ADMA presence may stimulate DDAH activity to maintain NO concentration on the stable level. Of course, many other factors may also influence NO/ADMA system e.g. methyltransferases activity responsible for arginine methylation and ADMA synthesis [16, 17] or the activity of proteins transporting ADMA into cells. The fact that liver cells damage, includ-

ing sinusoidal endothelial cells, with concomitant increase in their permeability is observed during the perfusion should also be taken under consideration [43].

SOD is an important antioxidative enzyme with great physiological significance. It catalyzes the conversion of single electron reduced species of molecular oxygen to hydrogen peroxide and oxygen [44]. Much data indicates protective properties of quercetin against oxidative stress in rat liver induced by carbon tetrachloride (CCl_4) [45], or ethanol [46] or reversed pro-oxidant effects of galactose-induced [47] or streptozotocin-induced [7] hyperglycaemic oxidative stress in rat. Less data is available for NaQSA. In present authors' previous work they have demonstrated that NaQSA significantly restored SOD activity in mice liver in subacute cadmium intoxication model but they did not observed any changes in liver SOD activity in groups of healthy mice [48]. In the present work no significant differences in SOD activity in groups perfused with NaQSA as compared to control group was noted. However, the effect of NaQSA on SOD activity depended on the concentration: the significantly higher SOD activity was observed in the group with 50 μM of NaQSA as compared to the group with 10 μM of NaQSA. No significant changes in SOD activity in Q10 and Q50 groups compared to control group may suggest that NaQSA in these concentrations did not protect rat liver and/or SOD activity could raise in response to oxidative stress. Nevertheless, oxidative stress may develop without changes in SOD activity and the level of this enzyme may be not directly connected with intensity of stress oxidation. Moreover, changes in SOD activity may be not strictly linked with changes in ADMA level and *in vitro* experiment showed that SOD activity needed to reverse the effects of ADMA may be much higher than changes in SOD evoked by ADMA [49] so the causal relation between their changes are very difficult to assessment. Because there is no available data about the plasma levels of NaQSA in rats after oral or parenteral administration and there are also no earlier *ex vivo* studies with quercetin or NaQSA, present results could not be supported by other evidence. The authors found it difficult to compare present results with those obtained *in vivo*.

A possible protective effect of NaQSA on liver function, expressed especially at lower values of AST, was observed for both studied concentrations. However, contrary to the effect of NaQSA on SOD or DDAH/ADMA, this compound at higher concentration (50 μM) exhibited the strongest protective effect, which was indicated by lower increase in AST activity between the 15th and 90th minute of perfusion. In many published studies, quercetin also protected liver function against CCl_4 toxicity [23], epirubicin [50], chronic cadmium intoxication [51]. A protective effect was also reported in fibrosis, biliary cirrhosis, alcoholic disease, ischemia-reperfusion (I/R) injury, and after acute chromium trioxide intoxication [21, 22, 52]. Analyzing all the works cited above, it could be noticed that quercetin does not exert any noticeable effect in healthy liver, but preserves liver function after it is exposed to several noxious factors. In present experiment the longer time of perfusion the stronger protective action of NaQSA was observed.

In summary, the results of the present study may suggest a protective effect of NaQSA on liver function expressed by lower aminotransferases activity, but it could be plausible that the impact of this compound on SOD activity and ADMA/ /DDAH system is not so evident and depends on NaQSA concentration. In lower concentration, NaQSA exerted some beneficial properties: increases DDAH activity and decreases ADMA concentration. But in higher concentration, the protective action of NaQSA is vanished: DDAH is decreased and ADMA is elevated. Not elevated level of SOD revealed in the present study needs further studies. To authors' knowledge it is the first study evaluating influence of NaQSA on ADMA/DDAH activity. Further detailed studies, especially in *in vivo* model, with different doses of NaQSA are necessary to assess the action of this compound on this field.

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