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Original Research Article

Investigating the influence of taurine on thiol antioxidant status in Wistar rats with a multi-analytical approach☆

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ABSTRACT

Taurine (2-aminoethanesulfonic acid) is an organic acid widely distributed in animal tissues. It is involved in many physiological processes. Thus, it is widely discussed especially due to its antioxidant properties. In this study, we focused on the effect of taurine supplementation on the concentration of antioxidants in blood plasma and erythrocytes of Wistar rats. Taurine was applied in feed mixture in the dosage of 0, 1, 250, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 mg/kg. We monitored both enzymatic and non-enzymatic antioxidants – glutathione peroxidase, glutathione reductase, and superoxide dismutase and reduced/oxidized glutathione and metallothionein. Using three different methods 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Ferric Reducing Antioxidant Power (FRAP) and free radicals, we determined antioxidant capacity. In addition, we monitored levels of uric acid and glucose. Our results revealed significant changes in both enzymatic and non-enzymatic parameters with the increasing taurine supplementation.

Introduction

Taurine is present in the majority of the human tissues in low concentrations (Awapara, 1950; Roe and Weston, 1965; Davison and Kaczmarek, 1971; Baskin and Dagirman, 1973; Fukuda et al., 1982; Sturman, 1993; Bouckenooghe et al., 2006). In addition, it occurs in foodstuffs, especially in seafood (Silva et al., 2011) and meat (Lau et al., 1990; Schmid, 2009). Moreover, taurine has significant anti-inflammatory

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properties (Marcinkiewicz, 2009) and participates in different physiological processes as it stabilizes cell membranes (Condron et al., 2010), regulates fatty tissues metabolism (Ueki and Stipanuk, 2009) and levels of calcium ions in blood (Ribeiro et al., 2010). As it follows from these facts, taurine is important in the prevention of cardiovascular diseases (Huxtable and Bressler, 1974; Hayes et al., 1975; Huxtable and Chubb, 1977; Thurston et al., 1981; Pion et al., 1987), obesity, type II diabetes and cancer (Zulli 2011; Caletti et al., 2012; Das and Sil, 2012; Feng et al., 2012; Giles et al., 2012; Ribeiro et al., 2012; Taranukhin et al., 2012; Zinellu et al., 2012). Due to biological functions of taurine, this compound is, moreover, intensively studied due to its possibility to regulate oxidation stress (Aruoma et al., 1988; Gurer et al., 2001; Hanna et al., 2004; Ghosh et al., 2009; Marcinkiewicz, 2010; Srivastava et al., 2010; Wu and Prentice, 2010). Protective role of taurine against oxidative stress in murine erythrocytes has been demonstrated (Sinha et al., 2008; Zitka et al., 2010; Hynek et al., 2012a). Treatment with taurine before cadmium intoxication prevented the toxin-induced oxidative impairments in the erythrocytes of the experimental animals (Sinha et al., 2008). Development and function of skeletal muscle (Ishikura et al., 2011; Ito and Azuma, 2011; Silva et al., 2011), the retina (Zeng et al., 2009) and the central nervous system (Wu and Prentice, 2010) may be regulated by taurine. Beside metabolic regulation, taurine also plays an important role in innate immunity (Nagl et al., 2000) and is directly related to antioxidant properties in clinical (Zulli, 2011; Shivananjappa and Muralidhara, 2012), toxicological (Turna et al., 2011; Yildirim and Kilic, 2011; Shao et al., 2012b) and oncological studies (Henderson et al., 2001; Gottardi and Nagl, 2010; Shalby et al., 2011; Das et al., 2012).

Based on the aforementioned papers investigating the impact of taurine on an organism, complex experiments allowing the understanding of the regulation of homeostasis during oxidative stress are still missing. For this reason, we aimed our experiment at the investigation of both enzymatic and non-enzymatic antioxidant systems, such as glutathione peroxidase, glutathione reductase, and superoxide dismutase and reduced/oxidized glutathione and metallothionein. Our work brings new knowledge about the role of taurine in homeostasis during oxidative stress. In addition, its supplementation is discussed with the oxidative stress itself. A wide scale of taurine doses was chosen to cover expected amounts taken by humans from food sources.

Materials and methods

Chemicals

Taurine and other chemicals were purchased from Sigma Aldrich Chemical Corp. (St. Louis, Missouri, USA), unless noted otherwise. The stock solution of 800 mM taurine was prepared by dilution with Milli Q distilled water. The deionised water was prepared using reverse osmosis equipment Aqual 25 (Czech Republic). The deionised water was further purified by using apparatus MiliQ Direct QUV equipped with the UV lamp. The resistance was 18 MΩ. The pH was measured using pH meter WTW inoLab (Weilheim, Germany).

Laboratory animals and their keeping

In total, 60 males of Wistar rats (Biotest, Konárovice, Czech Republic) were used in the experiment. At the beginning of experiment, the animals were 28 days old and differences in body weight were in a range ±2.5 g. The animals were kept in an air conditioned room with stable temperature of 23 ± 1 °C, humidity 60%, light period 12 h with light intensity up to 200 lx, content of CO_2 up to 0.25%, NH_3 up to 0.0025%. Food and water were provided ad libitum during the whole experiment. All animals were in good condition and no aberrations in behavior and development were observed. The experiment was approved and supervised by ethical committee, Mendel University in Brno, Brno, Czech Republic.

Experimental design

Animals were divided into 12 experimental groups (5 males in each group). They were supplemented with taurine through feed mixture, which was enriched by taurine in the dosage of 0, 1, 250, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 mg/kg per four weeks. Each animal consumed 35 g of feed mixture per day in average. Animals were put to death after 28 days. Blood sample was obtained by the puncture to heart into heparin-coated micro test tubes (Dispolab, Germany). Subsequently the centrifugation step followed (700 × *g*, 10 min) and components (erythrocytes, blood plasma) were analyzed.

Taurine determination

Preparation of erythrocytes sample

A sample (0.1 g of erythrocytes, fresh weight) was deeply frozen by liquid nitrogen. After it, 1 ml of 0.2 M phosphate buffer (pH 7.0) was added. Sample was vortexed for 15 min and centrifuged at 24,000 × *g* (20 min at 4 °C). A volume of 380 μl of supernatant was taken and mixed with 20 μl of 100% trifluoroacetic acid (TFA). Sample prepared like this was further centrifuged (24,000 × *g*, 20 min, 4 °C). After it, a volume of 237 μl was taken and it was mixed with 12.5 μl of 100% TFA. After centrifugation (24,000 × *g*, 20 min, 4 °C), supernatant (200 μl) was mixed with 200 μl of diluting buffer (thiodiglycol 5 ml/l, citric acid 14 g/l, sodium chloride 11.5 g/l, sodium azide 0.10 g/l). Finally, this sample was prepared for the analysis using the AAA 400 apparatus.

Preparation of blood plasma sample

A sample of blood plasma (380 μl) was mixed with 20 μl of 100% TFA.Sample prepared like this was further centrifuged (24,000 × *g*, 20 min, 4 °C), after it a volume of 237 μl was taken and it was mixed with 12.5 μl of 100% TFA. After centrifugation (24,000 × *g*, 20 min, 4 °C), supernatant (200 μl) was mixed with 200 μl of diluting buffer (thiodiglycol 5 ml/l, citric acid 14 g/l, sodium chloride 11.5 g/l, sodium azide 0.10 g/l). Finally, this sample was prepared for the analysis using the AAA 400 apparatus.

Taurine analysis

For determination of taurine, an ion-exchange liquid chromatography (Model AAA 400, Ingos, Czech Republic) with