UDC 577.29.577.151

EFFECT OF TAURINE ADMINISTRATION ON ACTIVITY OF SUPEROXIDEDISMUTASE IN RAT TISSUES

R. D. Ostapiv[1,2], V. V. Manko[1]
vvmanko@franko.lviv.ua

[1]Ivan Franko National University of Lviv, 4, Hrushevskyi St, Lviv 79005, Ukraine
[2]SSRCI of Veterinary Medicinal Products and Feed Additives,
11, Donetska St, Lviv 79019, Ukraine

Aim of work was to study the activity of superoxide dismutase and its isozymes in rat tissues under the conditions of prolonged oral administration of taurine. Male Wistar rats weighing 190–220 g and age 4.5 months, were divided into three groups. Rats for 28 days, daily, though esophagus probe were injected: control — drinking water and two experimantal — taurine solution of 40 (I experimental group) and 100 (II experimental group) mg/kg, respectively. After completing the research animals were decapitated under light chloroform anesthesia and liver, brain, testis and thigh muscle tissues were isolated. Tissues were homogenized, centrifuged. In supernatant was determined total superoxide dismutase and isozyme content after electrophoresis in 10% polyacrylamide gels, staining with tetrazolium nitroblue. Given the percentage isozyme, the activity of each was deducted from the total activity of the enzyme.

It was found that activity of brain superoxide dismutase remains within control under the conditions of prolonged per oral administration of taurine. However, cytoplasmic redistribution of isozyme activities occurs — S2 increases, and S3 and S1 decreases. The superoxide dismutase liver of I experimental group animals is the highest. This occurs because of the increase in activity of extracellular (S5) and cytoplasmic (S2) isozymes. With the increase of SOD activity in testes of II experimental group increases the quantity and value of all isozymes. In muscles of both experimental groups increased total activity, with it grow extracellular (S5) and cytoplasmic (S1) activity of superoxide dismutase isozymes.

Consequently, the impact of taurine long-term oral administration on activity of superoxide dismutase is realized through isozyme content change, and is tissue- and dosespecific.

Keywords: TAURINE, SUPEROXIDE DISMUTASE, ISOZYME, LIVER, BRAIN, TESTES, THIGH MUSCLE

АКТИВНІСТЬ СУПЕРОКСИДДИСМУТАЗИ ТА ЇЇ ІЗОЗИМІВ У ТКАНИНАХ ЩУРІВ ЗА ТРИВАЛОГО ПЕРОРАЛЬНОГО ВВЕДЕННЯ ТАУРИНУ

Р. Д. Остапів[1, 2], В. В. Манько[1]
vvmanko@franko.lviv.ua

[1]Львівський національний університет імені Івана Франка,
вул. Грушевського, 4, м. Львів, 79005, Україна
[2]ДНДКІ ветеринарних препаратів та кормових добавок,
вул. Донецька, 11, м. Львів, 79019, Україна

Метою роботи було вивчити активність супероксиддисмутази та ізозимів у тканинах за тривалого перорального введення таурину щурам. Самців щурів лінії Wistar масою 190–220 г і віком 4,5 місяці, ділили на три групи, яким протягом 28 діб, щоденно, через стравохідний зонд вводили: контрольній — питну воду, двом дослідним — розчин таурину з розрахунку 40 (I дослідна група) і 100 (II дослідна група) мг/кг маси відповідно. Після завершення досліджень тварин декапітували під легким хлороформним наркозом і виділяли тканини печінки, мозку, сім'яника та стегнового м'язу. Тканини гомогенізували, центрифугували і в супернатанті визначали загальну активність супероксиддисмутази та вміст ізозимів, після електрофорезу в 10 % поліакриламідному гелі, фарбуванням з нітросинім тетразолієм. Враховуючи відсотковий вміст ізозимів, активність кожного з них вираховували з загальної активності ензиму.

Встановлено, що за тривалого перорального введення таурину активність супероксиддисмутази мозку залишається в межах контролю. Однак, відбувається перерозподіл активностей

цитоплазматичних ізозимів — S2 зростає, а S3 та S1 знижується. Активність супероксиддисмутази печінки тварин I дослідної групи найвища, при цьому зростає й активність позаклітинного (S5) та цитоплазматичного (S2) ізозимів. Зі зростанням активності супероксиддисмутази сім'яників II дослідної групи, підвищуються й величини значень усіх ізозимів. У м'язах обох дослідних груп зі зростанням загальної підвищуються й активності позаклітинного (S5) та цитоплазматичного (S1) ізозимів супероксиддисмутази. Отже, вплив тривалого перорального введення таурину на активність супероксиддисмутази реалізується через вміст ізозимів, і є тканино- та дозоспецифічним.

Ключові слова: ТАУРИН, СУПЕРОКСИДДИСМУТАЗА, ІЗОЗИМИ, ПЕЧІНКА, МОЗОК, СІМ'ЯНИКИ, СТЕГНОВИЙ М'ЯЗ

АКТИВНОСТЬ СУПЕРОКСИДДИСМУТАЗЫ И ЕЕ ИЗОЗИМОВ В ТКАНЯХ КРЫС ПРИ ДЛИТЕЛЬНОМ ПЕРОРАЛЬНОМ ВВЕДЕНИИ ТАУРИНА

Р. Д. Остапив1, 2, В. В. Манько1
vvmanko@lnu.edu.ua

[1]Львовский национальный университет имени Ивана Франко,
ул. Грушевского, 4, Львов 79005, Украина
[2]ГНИКИ ветеринарных препаратов и кормовых добавок,
ул. Донецкая, 11, Львов 79019, Украина

Целью работы было изучить активность супероксиддисмутазы и изозимов в тканях при длительном перорального введения таурина крысам. Самцов крыс линии Wistar массой 190–220 г и возрастом 4,5 месяца, делили на три группы, которым в течение 28 суток, ежедневно, через пищеводный зонд вводили: контрольной — питьевую воду и двум экспериментальным — раствор таурина из расчета 40 (I экспериментальная группа) и 100 (II экспериментальная группа) мг/кг массы, соответственно. После завершения исследований животных декапитировали под легким хлороформным наркозом и выделяли ткани печени, мозга, семенника и бедренной мышцы. Ткани гомогенизировали, центрифугировали и в супернатанте определяли общую активность супероксиддисмутазы и содержание изозимов, после электрофореза в 10% полиакриламидном геле, покраской с нитросиним тетразолием. Учитывая содержание изозимов, активность каждого из них вычисляли с общей активности фермента.

Установлено, что при длительном пероральном введении таурина активность супероксиддисмутазы мозга остается в пределах контроля. Однако, происходит перераспределение активностей цитоплазматических изозимов — S2 растет, а S3 и S1 снижается. Активность супероксиддисмутазы печени животных I экспериментальной группы самая высокая, при этом возрастает и активность внеклеточного (S5) и цитоплазматического (S2) изозимов. С ростом активности супероксиддисмутазы семенников II экспериментальной группа, повышаются и величины значений всех изозимов. В мышцах обеих экспериментальних групп с ростом общей повышаются и активности внеклеточного (S5) и цитоплазматического (S1) изозимов супероксиддисмутазы. Следовательно, влияние длительного приема внутрь таурина на активность супероксиддисмутазы реализуется через содержание изозимов, и есть ткане- и дозоспецифичным.

Ключевые слова: ТАУРИН, СУПЕРОКСИДДИСМУТАЗА, ИЗОЗИМ, ПЕЧЕНЬ, МОЗГ, СЕМЕННИКИ, БЕДРЕННАЯ МЫШЦА

Free oxygen radicals (FOR) are involved in many cell physiological processes. However, an increase in FOR contents destroys and damages cell membranes, organelles and DNA structure, accelerates necrosis and apoptosis [1]. Supports optimal physiological level and prevents excessive FRO formation in the organism the antioxidant defense system, including its enzymatic link [2]. Among the antioxidant defense enzymes the first in the transformation of oxygen free radicals is superoxide dismutase (SOD) — function of which is conversion of superoxide anion ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) [3]. During low activity of SOD processes of lipid peroxidation are activated, which leads to various pathologies [4]. One of compounds that regulate the activity of superoxide dismutase is taurine [5]. In

particular, a single intravenous administration of taurine in concentration 50 mg / kg can lead to the increase in SOD activity in the damaged hemisphere, and to the decrease in content of lipid peroxidation products [6]. In testes of rats SOD activity increased after 60 days of oral administration of 1% taurine aqueous solution [7]. However, oral administration of taurine in concentration 100 mg / kg to rats for 30 days did not alter superoxide dismutase activity in liver and brain of animals [8]. Thus, the effect of taurine on SOD activity is ambiguous and requires detailed study. Since the effectiveness of antioxidant protection and destruction of O2 • — depends on the activity and content aim of work was to study the total activity of superoxide dismutase and its isozymes under the conditions of long term oral administration of taurine to rats.

Materials and methods

Male Wistar rats were used in the experiments. The rats were 4.5 months old and with body weight 190–220 g at the beginning of the experiments. Animals were randomly divided into three groups: one control group and two experimental groups. During a 28 day period, animals were daily injected in esophagus: control group — drinking water, experimantal group I and II solution of taurine (40 and 100 mg/kg of body weight, respectively). The animals were decapitated on 29th day under light chloroform anesthesia and liver, brain, testes and thigh muscle were extirpated. All animal experiments were performed in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and the law of Ukraine "On the protection of animals against cruel treatment".

Organs were homogenized using Potter-Elvehjem homogenizer at 4º C in a ratio of 1 g of tissue in 5 ml of homogenization solution. For homogenization were used such solutions: sucrose (250 mM) — for brain and testis [9], KCl (250 mM) — for thigh muscle [10], solution consisting of 250 mM sucrose, 1 mM EGTA, 10 mM HEPES (pH 7.2) — for liver [11]. The homogenates were centrifuged for 15 min at 2000 g. The supernatant was discarded and activity of superoxide dismutase with nitroblue tetrazolium was determined (λ_{abs}=540 nm; UI/mg of protein) [12]; protein content in tissues was measured by Lowry method (λ_{abs}= 750 nm) [13]. Content of SOD isozymes was determined after electrophoresis in 10% polyacrylamide gel shading by Beauchamp C. and Fridovich I. [14]. After shading gels were scanned and with the help of TotalLab software percentage of isozymes was determined. The activity of each of the identified isozyme (a_n) was calculated using the formula:

$$a_n = \frac{A \times [SODn]}{100\ \%},$$

where A — total superoxide dismutase activity (IU / mg of protein), [SODn] — the content of corresponding isozyme (%). Analysis of research results was conducted by variation statistics method using the software Microsoft Exel 2010. The results are shown as the average mean and the error (M ± m), difference probability data was calculated by two-sample equal variance Student's t-test for independent samples [15].

Results and Discussion

In brain tissue taurine acts as an activator of the nervous system [5]. Incubation of neurons with added taurine increases the activity of antioxidant enzymes [9]. In contrast to the published data, SOD brain activity in both experimental groups remained on the level of control (*table. 1*).

The study of SOD isozyme content in rat tissues (brain, liver, testes and thigh muscle) revealed five protein bands that had enzymatic activity: one — extracellular (Cu, Zn-SOD-E) and mitochondrial (Mn-SOD) and three cytosolic S1, S2 and S3 — Cu, Zn-SOD (*Fig. 1*).

SOD isozymes of studied tissues and organs, depending on the location in cells, characterize by approximately the same electrophoretic mobility, but differ in intensity and area bands of active protein enzyme. These features cause tissue specificity of isozyme content. In addition, the difference is found

Table 1

Effect of long-term peroral taurine administration on activity of superoxide dismutase tissue of rats IU / mg of protein (M ± m)

Tissue	Control, n = 3	Experiment	
		Group I, n = 5	Group II, n = 3
Brain	1.08±0.24	1.21±0.30	1.37±0.10
Liver	0.59±0.12	0.90±0.18**	0.54±0.10$^{\&}$
Testes	0.23±0.04	0.27±0.08	0.60±0.10$^{\#\#\&\&}$
Thigh muscle	0.70±0.27	1.11±0.34*	0.97±0.13#

Note: * — show significant difference experimental group I vs control, # — experimental group II vs control groups, & — experimental group I vs II (one icon — with P <0,05, two — with P <0,01, three — with P <0,001)

between the experimental and control groups. For example, in liver of I experimental group animals the activity of isozyme S1 decreased on 43.5% (table. 2).

In both research groups activity of S2 isozyme in 2–2,5 times increased, and the activity S3, conversely, decreased in 7–9 times.

The liver is an organ that is involved in the utilization of xenobiotics, most of which, above all, activate processes of lipid peroxidation [5]. Taurine in many studies is marketed as an antioxidant that is able to increase the efficiency of utilization of FOR [10].

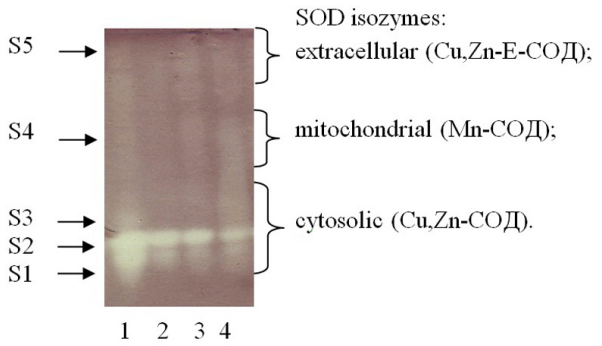

Fig. 1. Spectrum of SOD isozymes in rat tissues: 1 — brain; 2 — liver; 3 — testis; 4 — thigh muscle

Table 2

Effect of long-term peroral administration of taurine on activity of superoxide dismutase isozymes in rat tissues, M ± m

Tissue	Isozymes and their index number		Isozyme activity, UI/mg of protein		
			Control, n = 3	Experiment	
				Group I, n = 5	Group II, n = 3
Brain	Cu, Zn-E-SOD	S5	0.052±0.006	0.070±0.014	0.068±0.005
	Mn-SOD	S4	0.069±0.006	0.091±0.012	0.064±0.003
	Cu, Zn-SOD	S3	0.310±0.023	0.035±0.004***	0.050±0.004$^{\#\#\#\&}$
		S2	0.487±0.073	0.805±0.054**	1.060±0.043$^{\#\#\&\&}$
		S1	0.161±0.018	0.091±0.014*	0.126±0.012
Liver	Cu, Zn-E-SOD	S5	0.013±0.002	0.064±0.008***	0.037±0.010
	Mn-SOD	S4	0.014±0.003	0.018±0.006	0.020±0.004
	Cu, Zn-SOD	S3	0.033±0.005	0.045±0.028	0.020±0.004$^{\&}$
		S2	0.258±0.021	0.430±0.008**	0.314±0.020$^{\&}$
		S1	0.276±0.032	0.279±0.013	0.146±0.011$^{\#\&\&\&}$
Testes	Cu, Zn-E-SOD	S5	0.041±0.006	0.032±0.003	0.072±0.004$^{\#\#\&\&}$
	Mn-SOD	S4	0.027±0.009	0.030±0.002	0.056±0.006$^{\#\&\&}$
	Cu, Zn-SOD	S3	0.031±0.005	0.026±0.003	0.041±0.002$^{\&\&}$
		S2	0.464±0.029	0.497±0.040	0.745±0.035$^{\#\#\#\&\&}$
		S1	0.300±0.030	0.243±0.024	0.492±0.020$^{\#\#\&\&}$
Thigh muscle	Cu, Zn-E-SOD	S5	0.067±0.005	0.150±0.015**	0.145±0.012$^{\#\#\#}$
	Mn-SOD	S4	0.088±0.007	0.081±0.012	0.104±0.007
	Cu, Zn-SOD	S3	0.049±0.009	0.066±0.003	0.086±0.006
		S2	1.090±0.093	1.335±0.179	1.291±0.032
		S1	0.156±0.020	0.198±0.029**	0.247±0.012$^{\#\#}$

Indeed, in the liver of I experimental group animals SOD activity increased on 53 %, but indexes in the II experimental group remained at control level (see. Table. 1). The enzymatic activity increase in I experimental group animals lead to increased activity of extracellular S5 isozyme by almost five times and cytoplasmic S2 on 67% (see. Table. 2).

In testes tissue antioxidant defense enzyme activity is important because the growth of lipid peroxidation processes may affect spermatogenesis, DNA damage and loss of fertilizing ability by spermatozoa [4]. Drinking of 1% solution of taurine by rats for 60 days led to an increase in SOD activity in testes [7]. However, in the testes of I experimental group animals enzyme activity was on the control level (see Table. 1). Activity of isozymes remained also unchanged (see Table. 2). In testes of II experimental group animals SOD activity was nearly doubled and activity of all isozymes also increased.

Skeletal striated muscle is a tissue that largely requires energy in the form of ATP, which is synthesized in the mitochondrial electron transport chain. As a result of electron transport in mitochondria can form superoxide anion [10]. Because taurine can affect the activity of SOD, perhaps, increase its concentration in the tissue cells result in increased security of FOR [16]. Indeed, in the thigh muscle tissue of I and II research group animals SOD activity increased by 59 and 39% (see Table. 1). Thus, in animals of both experimental groups activity increased of extracellular S5 (124 and 117 %) and cytoplasmic S1 (27 and 58 %) isozymes (see Table. 2).

As a result, taurine long term peroral administration leads to increase in activity of SOD in liver of I experimental group, testes of II experimental group, and in thigh muscle of both experimental groups. Such an increase in enzymatic activity is based on changes in isozyme activity of Cu, Zn-dependent isozymes, both cytoplasmic and extracellular, but not mitochondrial Mn-dependent. It was shown that with increasing content of taurine increases the content of zinc in the brain of rats [5]. Cytoplasmic superoxide dismutase is Cu, Zn-dependent [3] and possibly taurine, indirectly, can increase the content of Zn^{2+}, and activates S2 and S1 isozymes in tissues of rats. Also the growth of total SOD activity and specific isozyme can point to a direct effect of taurine on the synthesis of SOD proteins, or an increase of lipid peroxidation processes in the testes tissue.

Conclusion

1. Activity of superoxide dismutase in brain of both I and II experimental groups animals are within control. However, the redistribution isozyme activity changes — S2 (Cu, Zn-SOD) activity rises.

2. Increase in superoxide dismutase activity in rat liver of I experimental group is due to rise in activities of extracellular isozyme (S5 Cu, Zn-SOD-E) and cytoplasmic (S2 Cu, Zn-SOD). The activity of the enzyme and its isozymes in the II experimental group is within the control.

3. The superoxide dismutase and its isozymes activity in I experimental group did not differ from control. In the testes of animals in II experimental group activity of all isozymes increased, this provided rise of total SOD activity.

4. In the thigh muscle of both experimental groups superoxide dismutase activity increased with rise of cytoplasmic (S1 Cu, Zn-SOD) and extracellular (S5; Cu, Zn-SOD-E) isozyme activity.

Perspectives for further research. It is expedient to investigate the respiratory activity of mitochondria in rat tissues at long-term administration of taurine.

1. Aruoma O. I., Halliwell. B., Hoey B. M., et al. The antioxidant action of taurine, hypotaurine and their metabolic precursors. *Biochem. J*, 1988, 256, pp. 251–255.

2. Yatabe Y., Miyakawa S., Miyazaki T. et al. Effects of taurine administration in rat skeletal muscles on exercise. *J. Orthop. Sci*, 2003, 8, pp. 415–419.

3. Zengin E., Sinning C., Zeller T. Activity of superoxide dismutase copper/zinc type and prognosis in a cohort of patients with coronary artery disease. *Biomark. Med*, 2015, 9(6), pp. 597–604.

4. Iwasaki A., Gagnon C. Formation of reactive oxygen species in spermatozoa of infertile men. *Fertil. Steril*, 1992, 57, pp. 409–418.

5. Huxtable R. J. Physiological Actions of Taurine. *Phys. rev*, 1992, 72, pp. 101–160.

6. Sun M., Zhao Y., Gu Y. et al Protective effects of taurine against closed head injury in rats. *J. Neurotr*, 2014, 20, pp. 1–9.

7. Young I. S., Woodside J. V. Antioxidants in health and disease. *J. Clin. Pathol*, 2001, 54, pp. 176–186.

8. Parildar-Karpuzoð H., Mehmetçik G., Özdemirler-Erata G., et al. Effect of taurine treatment on pro-oxidant-antioxidant balance in livers and brains of old rats. *Pharmac. Rep*, 2008, 60, pp. 673–678.

9. El Idrissi A. Taurine increases mitochondrial buffering of calcium: role in neuroprotection. *Am. Ac*, 2008, 34(2), pp. 321–328.

10. Dawson R., Biasetti M., Messina S., et al. The cytoprotective role of taurine in exercise-induced muscle injury. *Am. Ac*, 2002, 22, pp. 309–324.

11. Kondrashova M. N. Transaminase cycle of substrate oxidation in cell, as an adaptation mechanism to hypoxia. *Pharm. Correc. Hypox. St*, 1989, 1, pp. 51–70. (In Russian)

12. Dubinina E. E., Salnicova L. Ya., Yefimova L. F. Activity and izozyme spectrum of superoxide dismutase of erythrocytes. *Lab. work*, 1983, 10, pp. 30–33. (In Russian)

13. Lowry O.H., Rosebrough N. J., Fair A. L., et al Protein measurement with Folin phenol reagent. *J. Biol. Chem*, 1951, 193(1), pp. 264–275.

14. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem*, 1971, 44, pp. 276–287.

15. Derkach M.P., Gumenizkiy R.Ya., Chaban M.E. *Course of variational statistics.* Kiyv, High school, 1977. 210 p. (In Ukrainian)

16. Schaffer S. W., Azuma J., Mozzafari M. Role of antioxidant activity of taurine in diabetes. *J. Physiol. Pharmacol*, 2009, 87, pp. 91–99.