Amino Acids (2015) 47:1549–1558
DOI 10.1007/s00726-015-1995-0

ORIGINAL ARTICLE

Taurine increases testicular function in aged rats by inhibiting oxidative stress and apoptosis

Jiancheng Yang[1] · Xiaomeng Zong[1] · Gaofeng Wu[1] · Shumei Lin[1] · Ying Feng[1] · Jianmin Hu[1]

Received: 9 February 2015 / Accepted: 22 April 2015 / Published online: 10 May 2015

Abstract In males, the decline of androgen synthesis, spermatogenesis and sexual function are the main phenotypes of aging, which may be attributed to testicular dysfunction. Taurine can act as an antioxidant, a testosterone secretion stimulator, a sperm membrane stabilizer and motility factor, and an anti-apoptotic agent. Recent observational studies suggested that taurine may play an important role in spermatogenesis, but to date whether taurine has anti-aging effects on testes remains unknown. We found that in aged rats testicular SDH and G6PDH activities, marker enzymes of testes, serum testosterone, testicular 3β-HSD and 17β-HSD mRNA expression levels were significantly increased by taurine treatment. Taurine administration also markedly raised the sperm count, viability and motility, decreased the sperm abnormality. Our data suggested that taurine can postpone testicular function deterioration in aged rats. Importantly, we observed obvious elevation of testicular antioxidant enzymes (SOD, GSH, GSH-Px) activities, and remarkable reduction of ROS and MDA by taurine administration, indicating taurine can decrease testicular oxidative stress and lipid peroxidation in aged rats. Finally, we found taurine effectively reduced testicular DNA fragmentation, increased testicular Bcl-2 protein expression, and decreased cytochrome c, Bax, Fas, FasL and caspase-3 expression, suggesting taurine can prohibit aged testicular apoptosis by mitochondrial dependent and independent signal pathway. In summary, our results indicated that taurine can suppress testicular function deterioration by increasing antioxidant ability and inhibiting apoptosis.

Keywords Taurine · Increases testicular function · Oxidative stress · Apoptosis · Aged rats

✉ Jianmin Hu
hujiamin59@163.com

[1] College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110866, People's Republic of China

Introduction

Aging, either in men or male animals, is accompanied by reproductive dysfunction in which the main display of decline in steroidogenesis, spermatogenesis, sexual response and function and may be attributed to the testicular function deterioration (Schiavi and Rehman 1995; Levy and Robaire 1999; Zirkin and Chen 2000). Although the mechanisms responsible for the aging remain unclear, it is widely accepted that oxidative stress and apoptosis are the two major factors in the aging process (Harman 1981; Troen 2003; Harman 2001). The free radical theory of aging contends that oxygen free radicals, by-products of organism aerobic metabolism, specifically mitochondrial respiration, cause cumulative oxidative stress damage, which eventually results in aging (Harman 1983, 1992; Dröge 2003). Apoptosis is an important physiological process that has been associated with aging (Warner 1999; Higami and Shimokawa 2000), which can be triggered by both extrinsic and intrinsic signal pathways (Li and Yuan 1999). The extrinsic pathway for apoptosis involves Fas ligand (FasL), Fas receptor, Fas-associated protein with dead domain, the initiator caspase-8, the executioner caspase-3 and -7 that ultimately activate apoptosis (Nagata and Golstein 1995). In the intrinsic apoptotic pathway, the Bcl-2 family proteins play an important role, which consists of two functionally distinct protein groups, anti-apoptotic proteins (such as

Bcl-2 and Bcl-xL) and apoptotic proteins (for example Bax and Bad) (Green and Reed 1998; Chittenden et al. 1995). When Bcl-2 separates from the mitochondrial outer membrane, mitochondria membrane permeability will increase, which causes the release of mitochondrial intermembrane proteins including cytochrome c into cytoplasm where it binds with apoptotic protease-activating factor-1(Apaf-1) and caspase-9 to form the apoptosome that subsequently activates the executioner caspase-3, -6 and -7 (Pollack and Leeuwenburgh 2001; Liu et al. 1996; Cain et al. 2002). In the testis, apoptosis increase with age (Warner 1999), which may be attributed to the decrease of androgen levels (Steiner et al. 1984) and/or increase of oxidative stress in the tissue (Samanta et al. 1999; Sastre et al. 2000).

Taurine (2-aminoethanesulfonic acid), a conditionally essential amino acid, has been found to be the most abundant free amino acid in many tissues, and involved in various physiological functions including bile formation, osmoregulation (Schaffer et al. 2000), modulation of neurotransmission (Kuriyama et al. 1978), calcium binding and transporter regulation (Huxtable 1992), membrane stabilization (Huxtable and Bressler 1973), detoxification (Schaffer et al. 2000), antioxidation (Koyama et al. 1992) and essential roles in brain development (Sturman 1986). Taurine can be biosynthesized in the male reproductive system (Li et al. 2006; Yang et al. 2010b), and identified as the major free amino acid (Holmes et al. 1992; Lobo et al. 2000). In the testes, taurine immunoreactivity is specifically localized in the Leydig cells, vascular endothelial cells and interstitial cells (Lobo et al. 2000). In addition, taurine has been found rich in the sperm cells and seminal fluid (Hinton 1990; Holmes et al. 1992). Our previous studies have identified that taurine can stimulate testosterone secretion, increase sperm quality, enhance the sexual response and function in the aged rats (Yang et al. 2010a, 2013). Recent studies by Higuchi and his colleagues have confirmed that taurine may play an important role in spermatogenesis by protecting germ cells from oxidative stress (Higuchi et al. 2012). These results suggested that taurine may have a beneficial effect on testes. Further, several studies have shown that the protective effects of taurine on testes oxidative stress and apoptosis induced by heavy metals and some drugs (Aly and Khafagy 2014; Das et al. 2009, 2012; Manna et al. 2008). Accordingly, we hypothesized that taurine may increase testicular function in aged rats by elevating antioxidant ability and suppressing apoptosis.

The objective of this study was to determine the beneficial effects of taurine on testes in aged rats and decipher its mechanism. For this purpose, we set out to determine the effects of taurine and taurine depletion on testicular marker enzymes, androgen synthesis, sperm quality, anti-oxidative parameters and apoptotic related proteins expression in aged rats. We found that taurine could increase aged testicular testosterone synthesis and spermatogenesis functions. Additionally, testicular antioxidant ability was elevated by taurine treatment in aged rats. Furthermore, we identify taurine administration also could suppress age induced testicular apoptosis. The present results indicated that taurine can prevent testicular deterioration by its anti-oxidative and anti-apoptotic activities.

Materials and methods

Animals

Wistar male rats (20 months of age) were purchased from Kunming Institute of Zoology, Chinese Academic Sinica. After being acclimatized to the laboratory environment for 1 week, the animals were randomly divided into three groups ($n = 8$). Rats in the control group drank tap water, rats in β-alanine (β-Ala, taurine transporter antagonist) group drank water containing 1 % β-alanine (Sigma, USA), and rats in taurine group(Tau) drank water containing 1 % taurine (Sigma, USA). The level of taurine and β-alanine administration is based on our previous study. All rats were kept at 22 ± 2 °C with a 12 h/12 h light/dark cycle, and were allowed free access to rat diet and water. After 60 days, rats were euthanized, and then blood, testes and left cauda epididymides samples were collected. Blood samples were used for serum testosterone assay. One testis was homogenized and used for taurine and antioxidant parameters assay, and total RNA, DNA and protein were extracted from the other testis for real-time RT-PCR, DNA fragmentation analysis and western blotting, respectively. The cauda epididymides were treated for sperm quality assay.

Biochemical analysis

Testicular taurine content was analyzed by reversed-phase high performance liquid chromatography (HPLC) according to previous report (Shi et al. 2003). Serum testosterone (T) concentration was measured by ELISA using testosterone kit (Bioss, China) as per the manufacturer's protocol. The activities/levels of sorbitol dehydrogenase (SDH), glucose-6-phosphate dehydrogenase (G6PDH), superoxide dismutase (SOD), reduced glutathione hormone (GSH), glutathione peroxidase (GSH-Px), nitric oxide synthase (NOS), nitric oxide (NO), reactive oxygen species (ROS), catalase (CAT), malondialdehyde (MDA) and total protein were analyzed according to the instructions of respective reagent kit (Nanjing Jiancheng Bioengineering Institute, China).

Real-time PCR

Total RNA of testis was extracted using RNAiso Plus according to the procedure of the supplier (TaKaRa, China). RNA purity and quality were determined by spectrophotometry at 260 and 280 nm. AMV First Strand cDNA Synthesis Kit (Sangon, China) was used to synthesize cDNA from RNA. Real-time PCR was performed on a Bio-Rad iQTM5 system using SYBR Green PCR Master Mix (ABI). The primers were designed for the genes of interest: 3β-HSD (TGTGCCAG CCTTCATCTAC-forward and CTTCTCGGCCATCCTTT T-reverse), 17β-HSD (GACCGCCGATGAGTTTGT-forward and TTTGGGTGGTGCTGCTGT-reverse), and β-actin (TCGTGCGTGACATTAAAGAG-forward and ATTGCCG ATAGTGATGACCT-reverse). Melting curve was analyzed for all the reaction. The relative gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method and normalized to the expression of the housekeeping gene β-actin in the same sample (Schmittgen and Livak 2008). Data were presented as relative fold-change and compared with control group rats.

Sperm quality assay

The left cauda epididymides were finely minced in 4 mL isotonic saline of 35 °C and repeatedly blown with transfer-pettor to prepare sperm suspension. After incubating 10 m at 35 °C, sperm suspension was used to determine sperm quality according to reported methods (Türk et al. 2008). Sperm quality was determined by four parameters: sperm count, motility, viability and abnormality.

DNA fragmentation analysis

Total genomic DNA from testis was extracted and purified using an Apoptotic DNA Ladder extraction kit with spin column (Beyotime, China) in accordance with the manufacturer's protocols. Equal amounts of DNA were electrophoresed on a 1.2 % agarose gel. Then DNA fragments were stained, visualized and photographed by the Gel Doc XR system (Bio-Rad, USA).

Western blotting

Testis protein was extracted according to the instruction of whole protein extract kit (Applygen, China). Protein quantification was determined by using BCA protein assay kit (Applygen, China). Thirty micrograms of protein was fractionated on SDS-PAGE and transferred to a polyvinylidenefluoride membrane (Bio-Rad, USA). After blocking 2 h at room temperature with blocking buffer, membranes were incubated overnight at 4 °C with primary antibodies including anti-Bcl-2 (Santa Cruz, USA), anti-Bax (Santa Cruz, USA), anti-cytochrome c (Santa Cruz, USA), anti-Fas (Santa Cruz, USA), anti-FasL (Santa Cruz, USA), anti-caspase-3 (Sigma, USA), anti-caspase-8 (Sigma, USA), anti-caspase-9 (Sigma, USA). The membranes were washed in TBST for 1 h and incubated with appropriate peroxidase-conjugated secondary antibody (Santa Cruz, USA) for 2 h at room temperature. Specific signal was visualized by Super ECL kit (Applygen, China). The protein bands were quantified by Image Quant 5.0 software (Molecular Dynamics) and normalized to individual β-actin expression levels.

Statistic analysis

All the data were expressed as mean ± SE and significant differences were determined by one-way ANOVA and Duncan's multiple range test using SPSS 16.0 software. A difference is considered significant at the $P < 0.05$ level.

Results

Taurine administration elevates testicular taurine concentration

We first measured the effects of taurine and β-Ala treatment on testicular taurine content in aged rats. As shown in Fig. 1, taurine administration significantly increased taurine level in aged rat testes ($P < 0.01$), whereas, β-Ala treatment statistically decreased testicular taurine level ($P < 0.05$), which indicated that β-Ala administration resulted in testicular taurine depletion.

Taurine increases the activities of testicular marker enzymes

It has been reported that SDH and G6PD are important marker enzymes of testicular function (Hodgen and Sherins

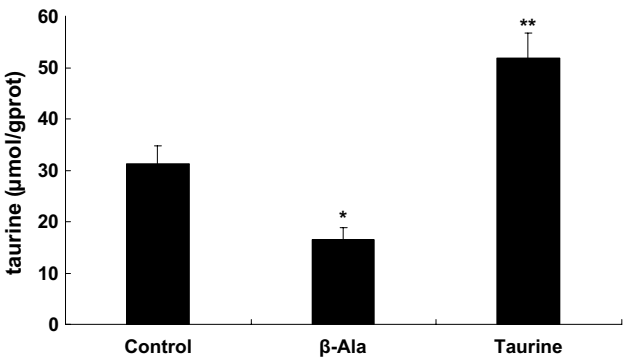

Fig. 1 Effects of taurine and β-Ala administration on testicular taurine concentration. Results are presented as mean ± SE ($n = 5$). *$P < 0.05$: significantly different from control group, **$P < 0.01$: significantly different from control group

1973; Prasad et al. 1995). We determined the effects of taurine and β-alanine (resulting in taurine depletion (Yang et al. 2010b)) on the activities of SDH and G6PD to identify the function of taurine in aged testes. The results (Fig. 2) showed that taurine depletion could statistically decrease the activities of SDH and G6PD in aged male testes compared to control group ($P < 0.05$), but taurine treatment could significantly increase the activities of the two testicular marker enzymes ($P < 0.05$). The experimental results suggested that taurine may prevent the deterioration of testicular function.

Taurine stimulates testosterone secretion

Androgen secreted by testicular Leydig cells plays an important role in male reproduction including maintenance of testes function. We measured serum testosterone concentration and mRNA expression levels of biosynthesized key enzymes for androgen, 3β-HSD and 17β-HSD, in aged rats. As shown in Fig. 3, levels of serum testosterone and testicular 3β-HSD and 17β-HSD mRNA expression were statistically decreased by taurine depletion in comparison with the control ($P < 0.05$), indicating taurine may be essential for androgen synthesis and secretion. However, treatment with taurine significantly elevated these three parameters compared to the control rats ($P < 0.05$), which suggested that taurine could stimulate testosterone secretion by increasing testicular 3β-HSD and 17β-HSD mRNA expression in aged rats.

Taurine elevates the sperm quality

Spermatogenesis is one of the major functions of testes, which happen in the testicular seminiferous tubules and is regulated by androgen. So we hypothesized that taurine may enhance testicular spermatogenesis function. Figure 4 shows the effects of taurine and taurine depletion on the sperm quality of aged rats. The sperm count, viability and motility were markedly raised ($P < 0.05$), but the sperm abnormality was obviously decreased in taurine treatment compared with the controls ($P < 0.05$). Taurine depletion, however, could notably decrease the sperm count and motility ($P < 0.05$), and increase the sperm abnormality ($P < 0.05$). The present results suggested that taurine may prohibit testicular spermatogenesis dysfunction in aged rats.

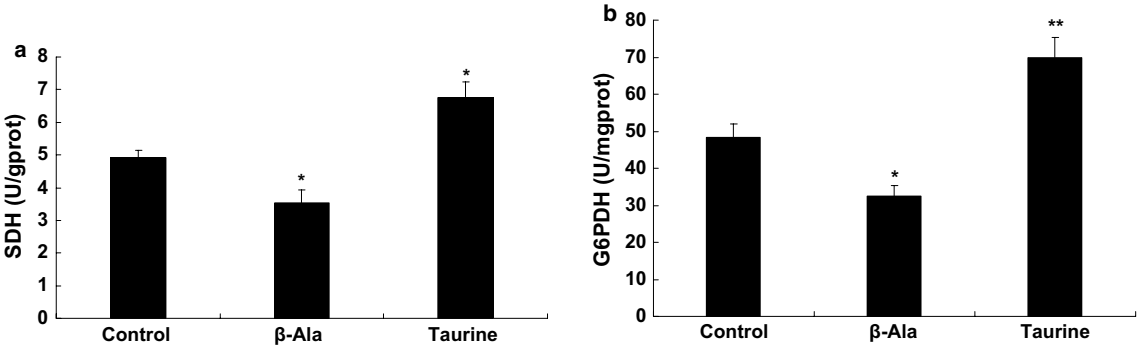

Fig. 2 Effects of taurine on the activities of testicular marker enzymes. Results are presented as mean ± SE ($n = 5$). *$P < 0.05$: significantly different from control group, **$P < 0.01$: significantly different from control group

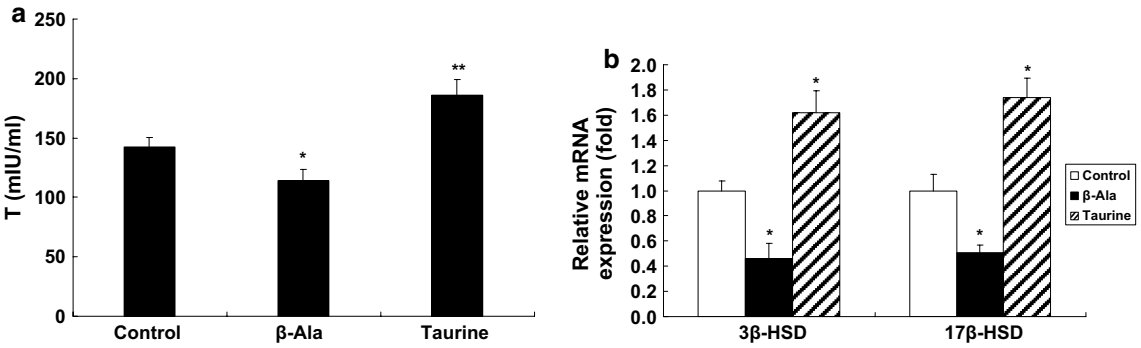

Fig. 3 Effects of taurine on androgen secretion in aged rats. Results are presented as mean ± SE ($n = 5$). *$P < 0.05$: significantly different from control group, **$P < 0.01$: significantly different from control group

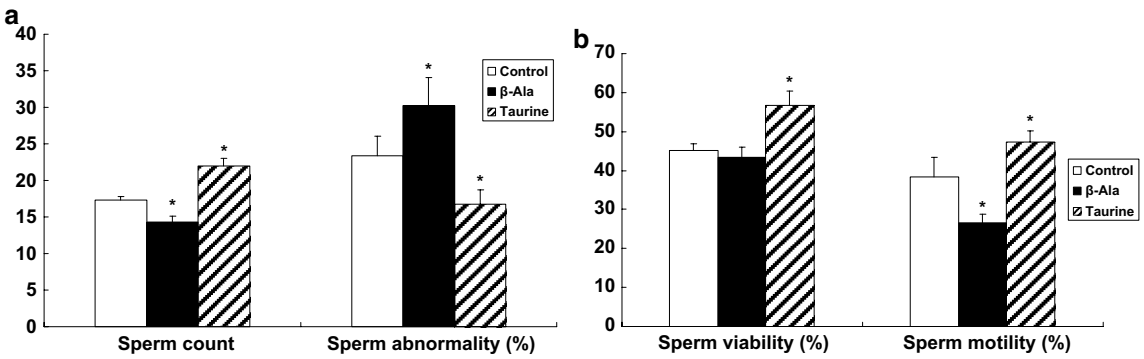

Fig. 4 Effects of taurine on the sperm quality in aged rats. Sperm count ($\times 10^6$/ml). Results are presented as mean ± SE ($n = 5$). *$P < 0.05$: significantly different from control group

Taurine enhances testicular anti-oxidative ability

It is well known that organism oxidative stress increases with aging. There have been several reports that suggest taurine acts as an antioxidant in vivo and in vitro (Aruoma et al. 1988; Yu and Kim 2009; Koyama et al. 1992). We, therefore, assayed the activities of SOD, GSH, GSH-Px, NOS, NO, ROS, CAT and MDA in the testes tissues of the experimental rats (Fig. 5). In taurine depletion rats, the levels of GSH, GSH-Px and CAT significantly decreased ($P < 0.05$), but the production of ROS and MDA statistically increased compared to the controls ($P < 0.05$). Taurine administration, nevertheless, remarkably increased testicular SOD, GSH, GSH-Px, NOS and NO activities ($P < 0.05$), and decreased ROS and MDA levels in aged rats ($P < 0.05$). These results indicted that taurine could reduce testicular oxidative stress in aged rats by increasing the activities of anti-oxidant system and decreasing the lipid peroxidation.

Taurine decreases testicular apoptosis

It has been identified that cell apoptosis will increase with age and is regulated by a family of related proteins associated with either the inhibition or augmentation of cell death, including Bcl, Fas and caspase family proteins, cytochrome c, et al. (Pollack and Leeuwenburgh 2001; Zhang and Herman 2002; Zhang et al. 2003). In the present study, we have examined the effects of taurine and taurine depletion on testicular apoptosis and related proteins expression in aged rat testes. The results (Fig. 6) showed that DNA ladder (an indication of apoptosis) was formed in the testicular genomic DNA of the control and β-alanine group rats, whereas, taurine administration effectively reduced the testicular DNA ladder in aged rats. Immunoblot analyses (Fig. 7) showed that taurine depletion stimulated the testicular expressions of Bcl-2, Bax, Fas, Fas-L, cytochrome c, caspase-3, -8 and -9 compared with the control rats, although these changes have no statistical difference ($P > 0.05$). Taurine treatment, however, significantly stimulated Bcl-2 protein expression ($P < 0.05$), obviously decreased the expressions of Bax, Fas, FasL, cytochrome c and caspase-3 ($P < 0.05$). These data demonstrated that taurine can prohibit aged testicular apoptosis.

Discussion

Aging is the progressive accumulation of changes with time which is related to or responsible for the ever-increasing physiological degeneration accompanying advancing age. In males, androgen decrease results in testicular dysfunction, a crucial example of aging (Labrie et al. 1997). It has been identified that taurine, an endogenous amino acid, has several roles in tissues/cells function, and can act as anti-oxidant (Aruoma et al. 1988), anti-apoptotic factor (Yalçınkaya et al. 2009) and testosterone stimulator (Yang et al. 2010b), which suggests that taurine may postpone testicular function deterioration. Therefore, unraveling the beneficial effects of taurine on aged testes and its' mechanism will not only lead to a better understanding of taurine's biological function but may also accelerate its' potential application in anti-aging.

To investigate the effects of taurine on the testicular function of aged rats, we first examined the effects of taurine and taurine depletion treatment on the changes of testicular SDH and G6PDH activities in aged rats, which are well known marker enzymes of testes function. Results showed that taurine depletion markedly decreased the activities of testicular SDH and G6PDH, while taurine administration effectively increased the activities of theses parameters. In the testes, SDH is widely distributed in the seminiferous tubules and germ cells, and related

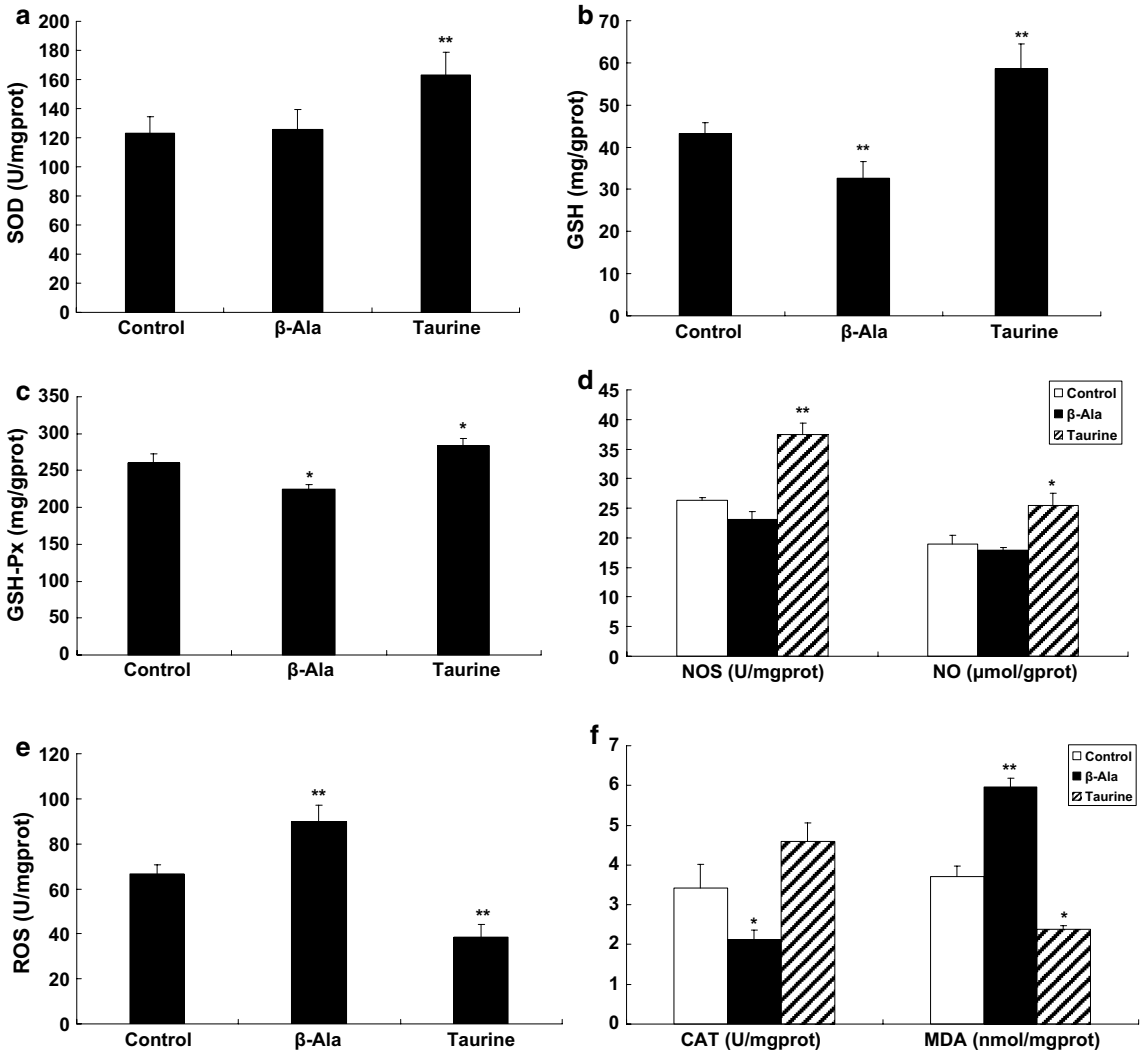

Fig. 5 Effects of taurine on testicular oxidative stress in aged rats. Results are presented as mean ± SE ($n = 5$). *$P < 0.05$: significantly different from control group, **$P < 0.01$: significantly different from control group

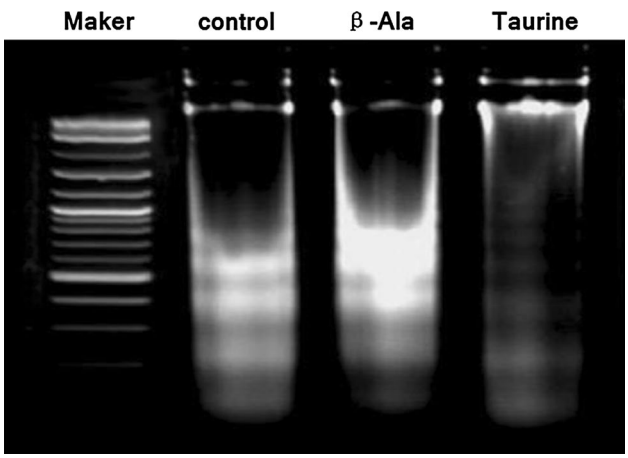

Fig. 6 Effects of taurine on testicular DNA ladder in aged rats. Marker: DNA marker (100–1000 bp). The experiment was repeated five times with similar results

to testes development, energy metabolism, maturation and spermatogenesis (Hodgen and Sherins 1973). Testicular G6PDH not only provides reducing equivalents in the process of androgen synthesis, but also decreases the activity of glutathione metabolism related enzyme which provokes oxidative stress and may result in cell death (Sinha et al. 1997; Mani et al. 2002). The present findings suggested that taurine is beneficial for aged testes function and antioxidative ability. The results are consistent with studies of Das and his colleague who reported that taurine could elevate testicular SDH and G6PDH activities in doxorubicin-induced testicular oxidative stress and apoptosis in rats (Das et al. 2012). It has also been demonstrated by Aly and Khafagy that taurine has the same effect on testicular G6PDH activity in endosulfan-induced rat testis oxidative stress and apoptosis (Aly and Khafagy 2014).

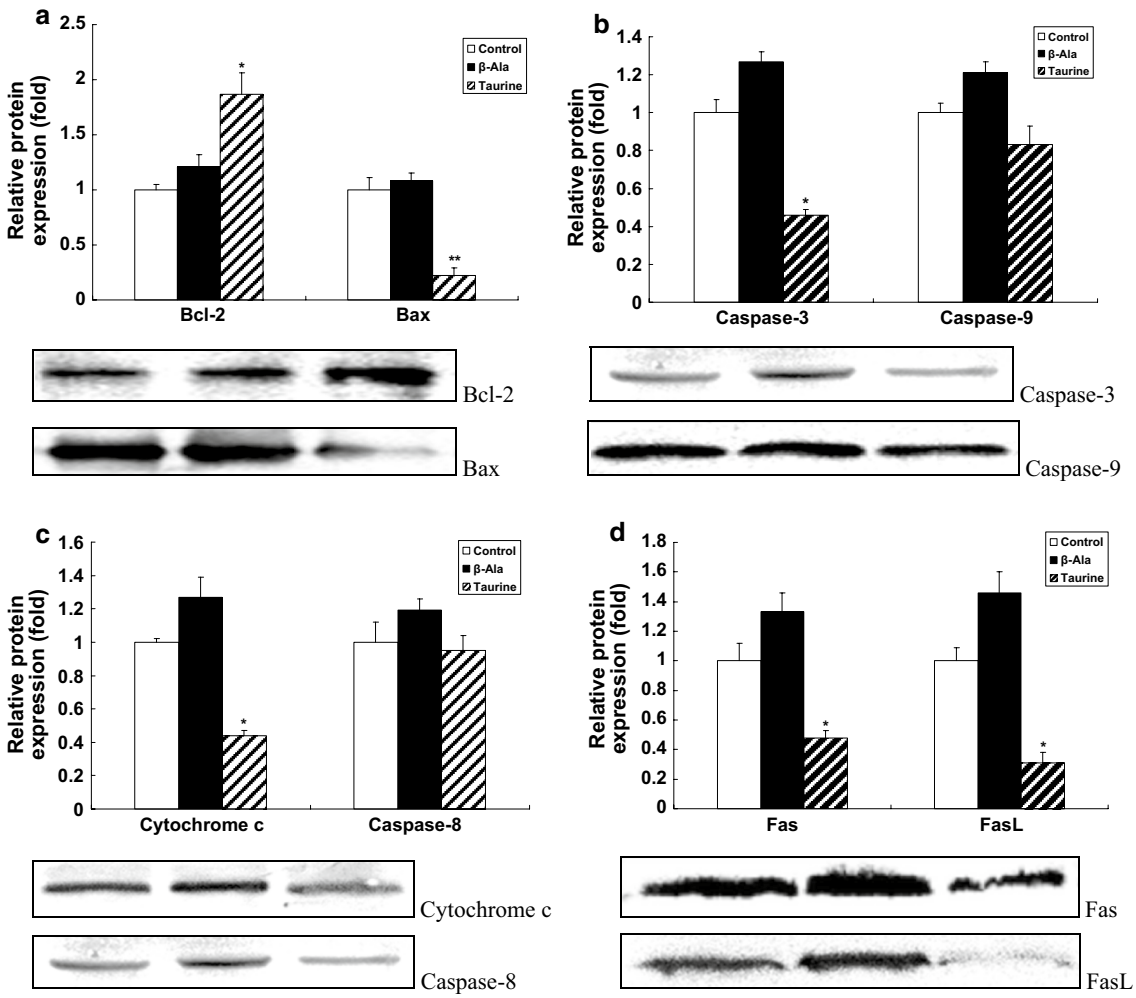

Fig. 7 Effects of taurine on the expression of testicular apoptosis-related proteins in aged rats. Results are presented as mean ± SE ($n = 5$). *$P < 0.05$: significantly different from control group, **$P < 0.01$: significantly different from control group

To validate whether taurine can ameliorate testicular androgen synthesis in aged rats, we analyzed the effects of taurine and taurine depletion on the level of serum testosterone and mRNA expression of testicular 3β-HSD and 17β-HSD, which are the key enzymes of androgen biosynthesis. Similar to our previous reports (Yang et al. 2010a, 2013), the testosterone concentration was significantly increased by taurine treatment, while taurine depletion has converse results. These data suggested taurine can stimulate testosterone secretion by increasing the expression level of testicular 3β-HSD and 17β-HSD. Our finding is also in accordance with the previous studies, despite that their animals had different treatment (Aly and Khafagy 2014; Das et al. 2009, 2012; Tsounapi et al. 2012).

To decipher the effects of taurine on the spermatogenesis in aged rat testes, sperm quality was determined in the present study. The results showed that sperm quality was markedly elevated by taurine administration in aged rats, which may be due to the stimulated effects of taurine on aged testicular testosterone, as high testosterone level is essential for seminiferous tubules, spermatogenesis and sperm maturation (Sharpe et al. 1992). In addition, it has been identified that taurine may prevent sperm lipid peroxidation (Alvarez and Storey 1983), act as a sperm capacitating agent (Meizel et al. 1980) and motility factor (Dröge 2003).

Taking the above results together, the present studies indicated that taurine indeed can postpone testicular functional deterioration, so we further investigated its mechanism. Over-produced free radicals, mainly ROS and reactive nitrogen species (RNS) by increasing oxidant stress with aging, is widely accepted as one of the important reasons for cellular senescence and organismic aging (Johnson et al. 1999). Excessive ROS not only stimulate consecutive reactions resulting in further free radicals production, but also induce lipid peroxidation that lead to producing lipid

peroxide (LPO) such as MDA, which then results in the damage of cellular compartments and function (Leutner et al. 2001). On the other hand, there is an antioxidant system involving SOD, GSH, GSH-Px and CAT which defend cellular integrity against free radical-induced damages by quenching oxidative elements (Irshad and Chaudhuri 2002). The present study demonstrated that taurine treatment increased the activities of SOD, GSH, GSH-Px and CAT in aged rat testes, and decreased the production of ROS and MDA. Alternately, taurine depletion reduced testicular GSH, GSH-Px and CAT levels, increased ROS and MDA production. Previous studies have reported that an excessive level of ROS may result in impaired steroidogenesis (Hanukoglu 2006) and spermatogenesis (Gupta et al. 2004). Our results suggested that taurine can increase the antioxidant ability of aged rat testes, which may be responsible for the amelioration of experimental rat testicular function. The beneficial effects of taurine on aged testicular anti-oxidative ability were possibly due to its direct and indirect antioxidant activities. As a direct antioxidant, taurine can neutralize and detoxify several free radicals and lipid peroxides (Aruoma et al. 1988; Cozzi et al. 1995). Taurine can also act as an indirect antioxidant by preventing oxidative stress induced permeability and stabilizing membrane permeability (Gordon et al. 1992; Timbrell et al. 1995). The present findings also showed that taurine could elevate testicular NO concentration by increasing the activity of NOS. The result is different from other previous studies, which identified taurine may inhibit the production of NO (Redmond et al. 1996; Gurujeyalakshmi et al. 2000). NO is a RNS that can induce cellular oxidant stress damage and apoptosis, however, in testes, it also involves an array of functions, including Sertoli cell tight junction dynamics, Leydig cell steroidogenesis, sperm motility and maturation (Zini et al. 1996; Lee and Cheng 2004). The reason for this discrepancy may be due to the difference of observed tissues, and the results indicated that taurine may ameliorate testicular androgen synthesis, spermatogenesis and sperm quality by NO pathway. In addition, it has been demonstrated that NO is a powerful vasodilator. The present results suggested that taurine can increase testicular circulation, which may be important for nutrition supply in androgen synthesis, spermatogenesis and sperm viability, and is also beneficial to the clearance of waste products including ROS.

Oxidative stress and excessive ROS are potent inducers of cell apoptosis, an active process of gene-directed self-destruction that could be completed through either mitochondrion-dependent or independent pathways. Furthermore, evidence has been accumulating to suggest that age-enhanced apoptosis may be contributed to age-associated changes such as progressive decline of physiologic function or disorders (Higami and Shimokawa 2000). Fas ligand/Fas receptor activation induced apoptosis is a classic example of mitochondrion-independent apoptotic pathway. The binding of Fas ligand and Fas receptor results in the recruitment of the adapter protein Fas-associated protein with death domain (FADD) and the initiator caspase-8 to form a death-inducing signaling complex, which in turn activate caspase-3 and -7 that are known as executioner caspase. In the present study, we found taurine administration could down-regulate Fas, FasL, caspase-8 and caspase-3 protein expression, suggesting anti-apoptotic effect of taurine involved in extrinsic pathway. In addition, mitochondria takes part in the initiation and regulation of the intrinsic apoptotic pathway, and the cross-talk with the extrinsic pathway in mammalian cells (Lee and Wei 2000, 2007). Release of cytochrome c into the cytosol from mitochondria regulated by the Bcl-2 family proteins is the primary event in mitochondrial apoptotic pathway, which leads to the formation of apoptosome and activation of caspase cascade (Li et al. 1997). It was found that the cytosolic level of cytochrome c and anti-apoptotic protein Bcl-2 were significantly decreased with age (Phaneuf and Leeuwenburgh 2002; Wang et al. 2001). Our results showed an up-regulation of Bcl-2, and down-regulation of apoptotic proteins cytochrome c, Bax and caspase-3 by taurine treatment, suggesting the involvement of intrinsic apoptotic pathway.

In conclusion, the present study suggested that taurine can increase testicular steroidogenesis and spermatogenesis in aged rats by increasing the activities of antioxidase system and decreasing the lipid peroxidation, and by its anti-apoptotic activity that implicated in mitochondrial dependent and independent signal pathway. These data provide important insights into the application of taurine in male anti-aging, although its exact mechanism remained to be further clarified.

Acknowledgments This work was supported by the National Natural Science Foundation of China (No. 30371048 and 31272522) and Program for Liaoning Excellent Talents in University (No. LJQ2014073).

Conflict of interest All authors state that they have no conflicts of interest, financial or otherwise.

Ethical approval All experimental protocols were carried out according to the guidelines of Shenyang Agricultural University Ethical Committee and in compliance to the Helsinki Declaration.

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