The Neuroprotective effects of taurine in toxicity induced by pyrazinamide in rat's brain

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Abstract: Pyrazinamide is a widely used drug for the treatment of tuberculosis (TB). Hepatotoxicity attributed to anti-TB drugs such as PZA is the most common of all adverse effect leading to PZA discontinuation in many patients using PZA. Although decades of use and large number of patients exposed to PZA worldwide, pathogenesis underlying toxicity is poorly understood in other tissue.

The results of many studies indicates the protective effects of taurine against several drug-induced multiple organ injuries in several animal models. Taurine, a conditionally essential amino acid, exhibits membrane stabilizing, and plays significant role as antioxidant to remove or inactivate free radicals as well as with several proposed roles in neurotransmission, and neuromodulation.

The primary objectives of this study were to investigate the mechanism of neurotoxicity induced by PZA in hippocampus. Apoptosis and oxidative stress process in hippocampus were evaluated. Also the protective effects of taurine as an antioxidant were studied.

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Introduction

Tuberculosis an infectious disease caused by mycobacterium tuberculosis, is an important problem in many countries of the world, especially in developing countries. Isoniazid, rifampin, pirazinamide and ethambutol are the first line drugs for the treatment of tuberculosis. Pyrazinamide (PZA) has significant effects in tuberculosis treatment and helps to shorten the course of the chemotherapy for the treatment of tuberculosis (Shi W et al. 2011; SH 2001). Unfortunately, a lot of side effects have been reported in patients receiving this medication, which in many cases leads to discontinuation of treatment in patients. The results of some studies indicate that PZA has a negative effect on the structure and function of chromosomes and can cause alter in nucleic acid structure and length (Anitha B et al. 1994; Kovalenko V et al. 2007). Hepatotoxic effects of pyrazinamide were demonstrated in several studies. The results of investigation showed that the metabolites of PZA, pyrazinoic acid (PA) and 5-OH-PA are the major toxic metabolites of PZA. Some findings express oxidative stress process are involved in hepatotoxicity induced by PZA and their metabolites(Adaramoye OA et al. 2016; Xin Liu et al. 2017). Cellular Toxicity induced by PZA and its metabolites in other tissues has not yet been studied. In our study the neurotoxic effects of PZA in hippocampus of rats was investigated (Tung-Yuan Shih C-YP et al. 2013.). Turin (2-amino-ethane sulfonic acid) the amino acid contains sulfur is present in most mammalian tissues such as the liver, heart, retina, brain and skeletal muscle(Batista TM et al. 2013; Lourenco R and M. 2002). Turin plays a major role in biological and physiological functions such as antioxidant, osmotic regulation, membrane stabilization and neurotransmission (SH 2001). The results of previous studies showed taurine reduce the level of ROS, inhibit lipid peroxidation and thus stabilize the biological membrane (Higuchi M et al. 2012; Wang 2013). The previous investigation showed the protective effects of turin on hepatotoxicity induced by many chemicals and drugs such as Phenytoin, carbamazepine, amitripryline, trazodone, isoniazid, hydrazine, iron and tamoxifen (Heidari R et al. 2013; Tabassum H et al. 2006; Hartman 2010; Liao 2008.; Taranukhin AG et al. 2008; Shohreh Taziki et al. 2013b; Shohreh Taziki et al. 2013a; Eghbal et al. 2013, 2014). Previous investigation have also showed that taurine has protective effects on cardiovascular diseases (Balkan J et al. 2002), complications of diabetes (SH 2001) and gastro-intestinal trauma (Çetiner M et al. 2005). The finding of some studies suggested that Turin has anti-apoptotic effects (Çetiner M et al. 2005; Takatani T et al. 2004; di Wu 1999). Also turin reduces the expression of Caspase 9 and 8 (key inducers of apoptosis) in mouse thalamic nuclei (Taranukhin AG et al. 2008; Waters E et al. 2001). However, the purpose of current research was to investigate the mechanisms of PZA induced toxicity in hippocampus of rats. We examined oxidative stress process in hippocampus. The levels of ROS

generation, Lipid peroxidation and reduced glutathione and apoptotic cells was investigated. Also, the protective roles of taurine in this cellular toxicity was investigated.

Methods & Materials

Pyrazinamide and Taurine were purchaced from Sigma-Aldrich chemical Co. 5-5 dithionitrobenzoic acide (DTNB) and Thiobarbitoric acide and Trichloro acetic acide were purchaced from Merck chemical co. The Kits for apoptosis and ROS generation were purchaced from Co-mybiosourse. All salts were purchaced from Merck chemical co. Male Sprague-Dawley rats (250–300 g) were housed in ventilated plastic cages with an environmental temperature of 21-23°c and 12h light photoperiod and 50-60% relative humidity. Animals were fed a normal chow diet and water ad libitum. The animals were handled and used according the animal handling protocol that approved by a local ethic committee in Gorgan University of medical sciences, Gorgan, Iran. Animals were randomly divided equally in to four groups of ten rats. In the normal control group (1), normal rats were orally administered 1 ml /kg (of body weight) of saline daily. In group2 the normal rats were received 1 ml/kg of 500 mg/kg taurine orally. In group 3 the rats received 1 ml/kg of 500 mg/kg PZA in saline. In the PZA and taurine group (4), normal rats were orally administered 1 ml/kg of 500 mg/kg taurine and 1 ml/kg of 500 mg/kg PZA in saline daily. Rats were treated daily for 28 days(Tung-Yuan Shih C-YP et al. 2013.). After 28 days all animals were anesthetized with intraperitoneal injection of ketamine & xylacin, and the brain tissue was isolated.

Glutathione content

The excised brains were immediately frozen at -70º c and analyzed for Glutathione (GSH) within 24 hours. Hippocampus was separated and then samples were homogenized in phosphate buffer 0.1M in PH= 7.4. Then 1ml of supernatant was added to 1ml of TCA 5% and centrifuged. GSH Contents were assessed by determination non-protein sulphydryl contents with the Ellman reagents. 0.1 ml of supernatant was added to 2ml of phosphate buffer and 0.4ml distilled water and 0.5 ml DTNB, then absorbance was measured at 412 nm using an Ultrospec 2000 UV spectrophotometer(Reza Heidari et al. 2015).

Lipid peroxidation

The levels of thiobarbituric acid reactive substances (TBARS) that were formed during the peroxidation of polyunsaturated fatty acids were measured. Brains were removed and hippocampus was isolated and then homogenized with cold KC1 (1.15%) to make a 10% homogenate. Then 3 ml of phosphoric acid 1%and 1 ml of TBA 0.6%aqueous solution was added to 0.5 ml of 10% homogenate tissue. The mixture was heated for 45 min on a boiling water bath. Then; 4 ml of n-butanol was added and mixed, after centrifugation the absorbance of n-butanol phase was measured at 535 nm using an Ultrospec 2000 UV spectrophotometer(Reza Heidari et al. 2015).

Ros formation

ROS generation was measured by ELISA technique. In the first step, the hippocampus extract was prepared (10 mg tissues in 100µl PBS) in accordance with the ELISA protocol kit. Samples were placed at room temperature for 30 minutes. Then 100 µl of HRP-conjugated reagent was added to all wells and placed at 37˚C for 60 minutes. In the next step. 50 µl of chromogen A and 50 µl of chromogen B solution were added to all wells and mixed in darkness for 15 minutes. Then, 50 µl of stop solution was added to all wells and mixed. Then, the OD was read at 450 nm by ELISA Reader and the concentration was calculated by calibration curve. Finally, the results were analyzed using SPSS software.

Apoptosis

The rats were anesthetized with ketamin & xylazine. Then their brains were separated and the hippocampus sections were prepared. The apoptotic cells were detected and measured by the terminal deoxynucleotidyl transferase mediated dUTP nick end-labelling (TUNEL) method, using an in situ c cell death detection kit (Co mybiosource), according to the manufacturers protocol. A photograph of each section was produced using a microscope (BX53, Olympus, Tokyo) and a DP73 digital camera under a magnification of 400. To measure the area density of the apoptotic cells, the images were transferred on to a computer. Using OLYSIA cell sence software (Olympus) then the cells was counted manually(Jahanshahi et al. 2013; Anitha B et al. 1994).

Results

The Effects of pyrazinamide on Apoptotic cells

The results indicated that the number of apoptotic cells in the pyrazinamide group was not notably different from the control group.

The Effects of pyrazinamide on ROS generation

As shown in Figure 1, Reactive oxygen substance was increased dramatically in the pyrazinamide group in compared with the control group, Pretreatment of rats with taurine decreased ROS formation caused by pyrazinamide significantly ($p < 0.05$).

The results of lipid peroxidation test

The levels of malondialdehide derivatives were increased in hippocampus after exposure to pyrazinamide. Taurine decreased the production of TBARS drastically ($p < 0.05$) (Figure 2).

As shown in Figure 3, the GSH levels in pyrazinamide treated rats were reduced drastically, and in the group that received taurine, the GSH content was markedly augmented ($p < 0.05$).

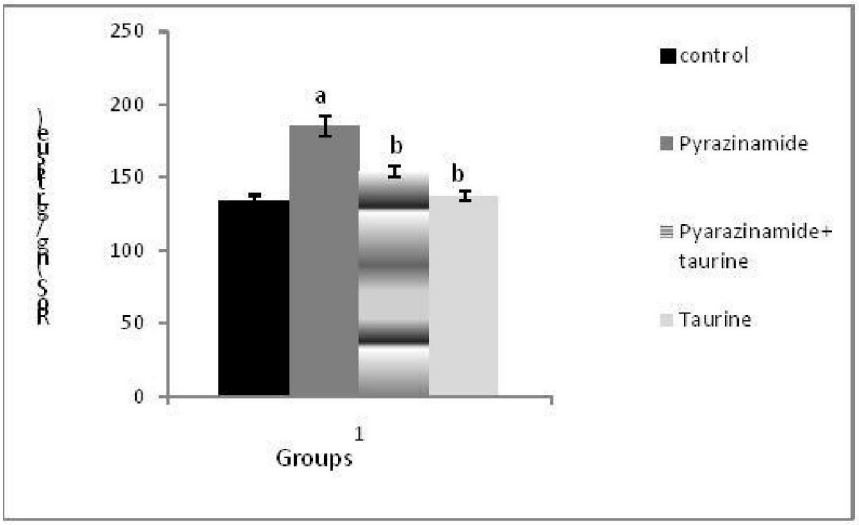

Figure1. Effect of Pyrazinamide alone and with Taurine in ROS formation in rat's hippocampus. Values are expressed as the mean±SED. a $p<0.05$, significant difference from control group; b $P<0.05$, significant difference from pyrazinamide treated rats.

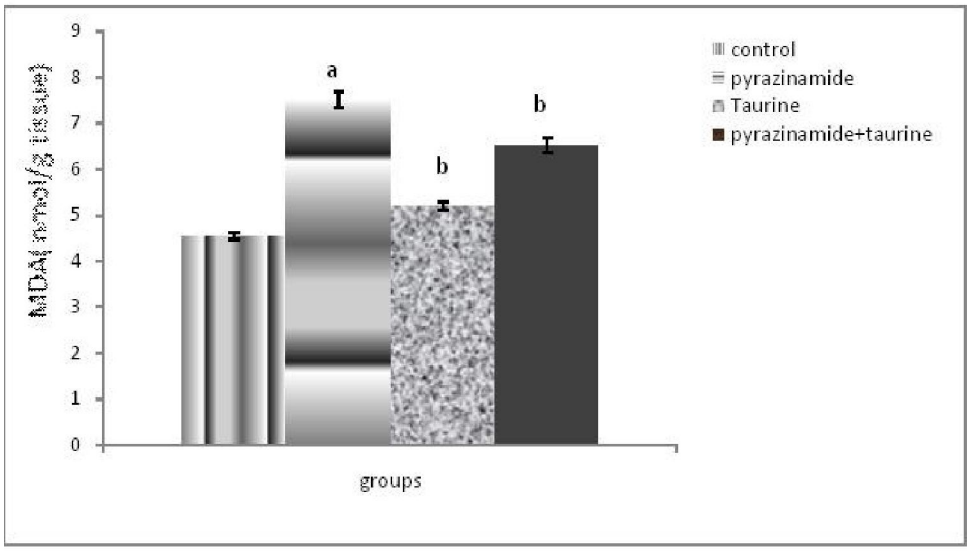

Figure 2. Concentrations of malondialdehyde (MDA) in rats' hippocampus. Data represent as means ± SEM. Level of significance p<0, 05. a significant difference from control; b significant difference from pyrazinamide treated group.

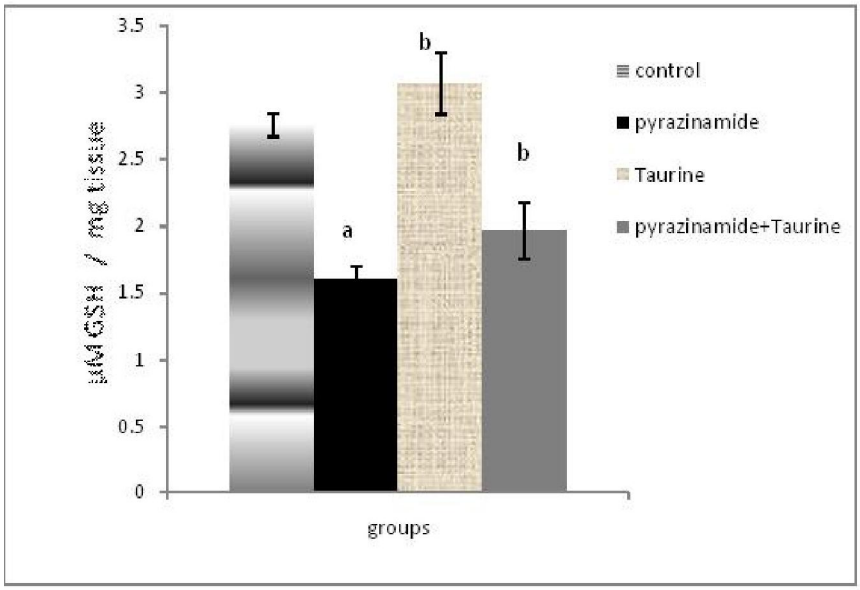

Figure 3. Effect of pyrazinamide alone and with taurine in the GSH levels in rats' hippocampus. Values are expressed as the means ±SED. a P?0.05 significant difference from control group; b P?0.05 significant difference from pyrazinamide treated rats.

Discussion

In current research, the biomarkers of oxidative stress such as ROS formation, lipid peroxidation, and the levels of protein sulfhydryls in pyrazinamide treated rat's hippocampus in the presence of an antioxidant (taurine) or without taurine was evaluated.

Our findings showed an increase in oxidative changes in the hippocampus, which, of course, did not significantly aggravate apoptosis.

Our results showed that ROS generation in hippocampus was significantly increased by pyrazinamide that supports the previous findings about pyrazinamide(Tung-Yuan Shih C-YP et al. 2013.).

Many finding about antituberculosis drugs were improved that oxidative stress is one of the important mechanisms of cytotoxicity induced by pyrazinamide and other anituberculosis agents. Our results are the new investigation about the mechanism of nerotoxicity induced by pyrazinamide and showed that oxidative stress was an vital pathway in pyrazinamide-induced cytotoxicity in hippocampus that is in accordance with earlier result (: El Idrissi; Anitha B et al. 1994; Chang KC et al. 2008).

Also, pyrazinamide neurotoxicity was prevented by taurine. ROS generation was drastically reduced in presence of taurine. As previously mentioned, taurine could be active as a potent radical scavenger (Batista TM et al. 2013; Çetiner M et al. 2005; Eghbal et al. 2013).

Present results indicate that taurine attenuated lipid peroxidation. Malondialdehyde derivative production in biological systems is usually one of the outcomes of ROS generation and oxidative stress (Eghbal MA et al. 2004). The effects of taurine in decreasing the lipid peroxidation induced by pyrazinamide might be due to its capacity of adjustment the oxidative stress induced by pyrazinamide that in accordance with earlier finding (Eghbal et al. 2013, 2014; Shohreh Taziki et al. 2013a; Shohreh Taziki et al. 2013b).

Reduced glutathione is the major endogenous antioxidant that neutralizes reactive oxygen substance. It is presence in most tissue and plays a vital role in protects living cells against oxidative stress [24].

GSH interfere with essential functions in living cells, including, scavenging free radicals, preserve the essential proteins, detoxifying electrophones, modulating important cellular function such as DNA synthesis (Eghbal et al. 2013).

Our finding improved that pyrazinamide motivate ROS formation and reduced GSH content in hippocampus cells. Earlier results improved that taurine could prevent GSH consumption by react with free radicals. Also it was supposed that taurine stimulated GSH synthesis (Eghbal et al. 2014, 2013). According to our results preventing the diminution of GSH could be a vital mechanism by which taurine attenuates the pyrazinamide-induced neurotoxicity. These finding comply with the previous results about taurine (Eghbal et al. 2013; Takatani T et al. 2004; SH 2001).

The results of other studies indicate that in the metabolism process, pyrazinamide produced a large amount of free radicals that is involved in the toxicity.

The results of this study confirm these findings because it was proved that the level of free oxygen radicals in the hippocampus is significant Increased.

Current results indicate that the administration of taurine had the potential of inhibiting oxidative stress and reducing the level of free radicals.

However, according to pathological reports, there was no clear difference in the incidence of apoptosis between control group and pyrazinamide group.

Due to that pyrazinamide is co-administrated with other anti-mycobacterium agents for a long time, it is recommended to investigated chronic neurotoxicity in future study.

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