Molecular and Cellular Biochemistry **277:** 131–135, 2005.

The effects of isoniazid on hippocampal NMDA receptors: Protective role of Erdosteine

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Received 28 February 2005; accepted 19 April 2005

Abstract

Isoniazid (INH) has neurotoxic effects such as seizure, poor concentration, subtle reduction in memory, anxiety, depression and psychosis. INH-induced toxic effects are thought to be through increased oxidative stress, and these effects have been shown to be prevented by antioxidant therapies in various organs. Increased oxidative stress may be playing a role in these neurotoxic effects. N-methyl D-aspartat receptors (NMDA) are a member of the ionotropic group of glutamate receptors. These receptors are involved in a wide variety of processes in the central nervous system including synaptogenesis, synaptic plasticity, memory and learning. Erdosteine is a potent antioxidant and mucolytic agent. We aimed to investigate adverse effects of INH on rat hippocampal NMDAR receptors, and to elucidate whether erdosteine prevents possible adverse effects of INH. In the present study, compared to control group, NMDAR2A (NR2A) receptors were significantly decreased and malondialdehyde (MDA), end product of lipid peroxidation, production was significantly increased in INH-treated group. On the other hand, administration of erdosteine to INH-treated group significantly increased NR2A receptors and decreased MDA production. In conclusion, decreasing NR2A receptors in hippocampus and increasing lipid peroxidation correlates with the degree of oxidative effects of INH and erdosteine protects above effect of INH on NR2A receptors and membrane damage due to lipid peroxidation by its antioxidant properties. (Mol Cell Biochem **277:** 131–135, 2005)

Key words: erdosteine, hippocampus, INH, MDA, NMDA

Introduction

Isoniazid (INH) still remains a first-line drug both for treatment and prophylaxis of tuberculosis [1, 2], but it is associated with neurotoxicity [3, 4]. Neurotoxic side-effects of tuberculosis chemotherapy occurred in 14.9% of patients with tuberculosis [5]. Incidence of acute INH neurotoxicity has been increasing because of the increasing incidence of tuberculosis [3]. INH remains among the five most common causes of drug-induced seizures in the United States [6]. Various studies have reported that INH treatment may be associated with headache and vertigo, sleep disorders, irritation, poor concentration, subtle reduction in memory, anxiety, depression and psychosis [5, 7]. Furthermore, INH caused encephalopathy in a case [8]. Severe acute INH neurotoxicity is characterized by a clinical triad including generalized seizures, coma, and metabolic acidosis [3]. Ingestion of INH of 80–150 mg kg^{-1} produces severe central nervous system symptoms [4, 9, 10]. The dose of INH that can cause convulsions is variable and may occur with ingestion of 40 mg kg^{-1} or less [11].

The mechanism of its toxicity is still unclear; however, several possible mechanisms were proposed [2]. Recent

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investigations have proposed that oxidative stress as one of the mechanisms responsible for INH-induced cytotoxicity and enhancement of protective mechanism by support to the thiols and antioxidant enzymes is likely to be an important adjunct in INH therapy [2].

Erdosteine, a homocysteine-derived mucolytic drug, has been successfully applied as a protective substance against various toxic agents such as acetaldehyde, adriamycin, bromobenzene, cyclophosphamide, halothane, isophosphamide, paracetamol and penicillic acid in animals and humans [12, 13]. Erdosteine metabolite (M1) contain free SH, therefore the free radical scavenging activity of M1 has attracted much attention by many research groups in recent years [14].

To the best of our knowledge, the possible protective effects of erdosteine on INH-induced possible toxic effects of N-methyl D-aspartat receptors (NMDAR) have not yet been investigated. Therefore, we aimed to investigate INH-induced possible toxic effects on NMDAR as well as the protective effect of erdosteine against INH neurotoxicity.

Materials and methods

Animals and treatment

Twenty-four male Wistar albino rats weighing between 215–255 g were divided into three experimental groups, with eight animals in each group; Control, INH-treated group, INH plus erdosteine-treated group. INH- and INH plus erdosteine-treated groups were treated orally with dose of $50\,mg\,kg^{-1}\,day^{-1}$ of INH [15–18] with the tap water for 15 days. Control group was given plain tap water. INH was obtained from Isparta Tuberculosis Dispansery (Isparta Tuberculosis Dispansery, Isparta, Turkey), dissolved in tap water, and orally administered for 15 days at a dose of $50\,mg\,kg^{-1}\,day^{-1}$ via plastic disposable syringes. Erdosteine was obtained from İlsan-İltaş Inc (İlsan-İltaş Inc, İstabul, Turkey), and orally administered for 15 days at a dose of $10\,mg\,kg^{-1}\,day^{-1}$ via plastic disposable syringes. The first dose of erdosteine was given 24 h prior to INH ingestion and continued until sacrifice. Equal amounts of vehicles instead of all drugs were given to the rats of control and INH-treated groups. After all the rats received the above treatments they were fed *adlibitum* until the midnight. Rats were anaesthetized with ether and tissue samples were obtained.

We hereby declare that the experiments reported here comply with the current laws and regulations of the Turkish Republic on the care and handling of experimental animals.

Malondialdehyde assays

After sacrificing the animals, the brain was removed and both hippocampi were then dissected out, washed in ice-cold phosphate buffered saline (PBS) and frozen immediately in deep freezer until further use. One of them was homogenized (1/10, w/v) in a glass-teflon homogenizer on ice-cold buffer (0.05 M potassium phosphate buffer, pH = 7.8). The homogenate was centrifuged at 10,000 × g for 15 min at 4 °C and used for the determination of malondialdehyde (MDA) concentration.

MDA, an end product of lipid peroxidation, was assayed by the method of Drapper and Hadley [19]. Protein in tissue homogenate was assayed by the Lowry's method [20].

Antibodies and chemicals

Anti-glutamate receptor NMDAR2A (NR2A), antiglutamate receptor NMDAR2B (NR2B), monoclonal-antirabbit IgG alkaline phosphatase conjugate, prestained molecular weight marker kit, nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (BCIP/NBT), leupeptin, aprotinin, benzamidine, ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) were purchased from Sigma (St Louis, MO, USA). All reagents were of analytical grade or the highest grade available. Antibodies against NR2A and NR2B were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.).

Western blot analyses

The other hippocampi were homogenized (1/10, w/v) in ice-cold buffer (50 mM Tris-HCl (pH 7.5), 0.15 M NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 25 μg/ml leupeptin, 25 μg/ml aprotinin and 10 μM benzamidine) and an aliquot was taken for protein determination by the Lowry 's method [20]. Equal amounts of protein for each sample (20 μg of protein per lane) were separated by SDS/PAGE on 7.5% minigels, blotted electrophoretically to PVDF membrane (Immobilon P), and incubated in Tris-buffered saline with Tween 20 (TBST) (50 mM Tris HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween 20) containing 3% bovine serum albumin for 30 min. Blots were incubated overnight with anti-NR2A (1:3000) or anti-NR2B (1:5000) in 1% BSA. Blots were then subjected to three additional 10-min washings in TBST. Blot were incubated with alkaline phosphatase-conjugated monoclonal anti-rabbit IgG (1:10 000) in 1% BSA for 1 h at room temperature and three additional 10-min washes carried out with TBST. The membrane was incubated in 20 ml of fresh reagent solution (BCIP/NBT) until color development. Images of immunoblots were analyzed with a computerized image analysis system (Uviphoto MW V.99, Ultra-Violet Products Ltd, Cambridge, UK). SDS-PAGE and Western blot analyses were done on three independent hippocampus preparations (2–3 animals/group). Size marker is indicated

on the left (myosin, 205 kDa). We examined the changes of INH on the protein concentration of NR2A and NR2B and whether erdosteine ameliorates INH induced changes. Western blot analysis was used to determine the changes of protein levels. The densities of protein bands in control groups were accepted as 100% and data from both INH- and INH plus erdosteine-treated groups were calculated as percentages of the control value.

Statistical analysis

Statistical analysis was carried out using the SPSS (Statistical Package for Social Sciences) version 11.0. The results are expressed as mean ± standard deviation. Groups were compared by Kruskal Wallis ANOVA Test. Statistical difference between two groups was calculated using Bonferroni corrected Mann–Whitney U-test.

Results

The level of hippocampal NR2A protein in INH-treated group was significantly lower than the control group ($p = 0.032$) (Fig. 1) and the level of NR2A in INH plus erdosteine-treated group was also significantly higher than the INH-treated group ($p = 0.001$) (Fig. 1).

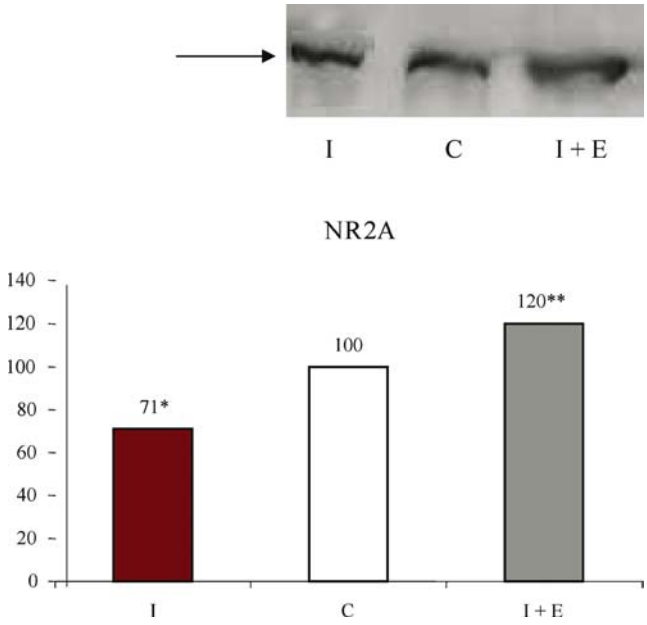

Fig. 1. Representative Western blot of NR2A in hippocampus from three groups of rats. C: Control; I: Isoniazid receiving group; I + E: Isoniazid and erdosteine receiving group. * $p = 0.032$, as C group is compared with I group. ** $p = 0.001$, as I group is compared with I + E group.

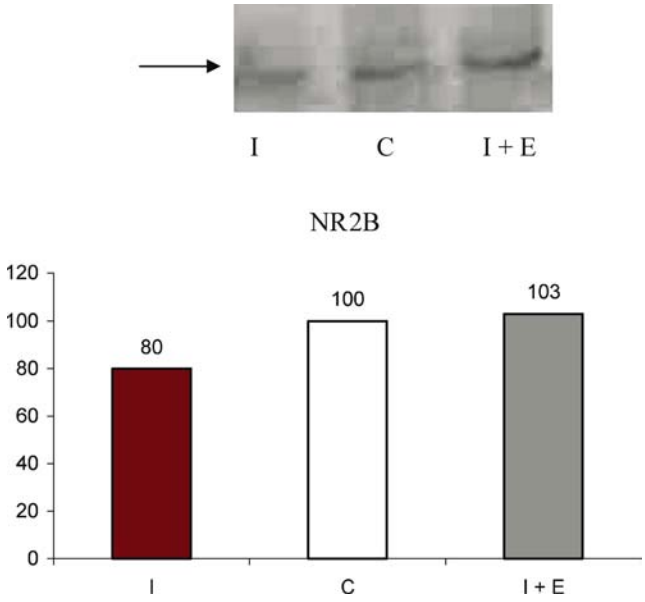

Fig. 2. Representative Western blot of NR2B in hippocampus from three groups of rats. C: Control; I: Isoniazid receiving group; I + E: Isoniazid and erdosteine receiving group.

The level of hippocampal NR2B protein in INH-treated group (80%) was not significantly lower than the control group (100%) and the level of NR2A in INH plus erdosteine-treated group was significantly higher than the INH-treated group ($p = 0.001$) (Fig. 2).

Compared to control group, MDA level was significantly increased ($p = 0.003$) in INH-treated group. Compared to INH-treated group, MDA level was significantly decreased ($p = 0.001$) in the INH plus erdosteine-treated group (Table 1).

Discussion

NMDAR is a member of the ionotropic group of glutamate receptors. Three kinds of NMDAR subunits: NMDAR1 (NR1), NMDAR2 (NR2) A-D, and NMDAR3 (NR3) have been identified over the past decade. Whereas NR1 is essential for

Table 1. Comparison of the MDA concentrations (values are mean ± S.D. for eight rats in each group)

	C	I	I + E
MDA (nmol/mg protein)	0.66 ± 0.09	0.88 ± 0.11*	0.61 ± 0.10**

MDA: Malondialdehyde; C: Control group; I: Isoniazid receiving group; I + E: Isoniazid and erdosteine receiving group.
* $p = 0.003$, as C group is compared with I group.
** $p = 0.001$, as I group is compared with I + E group.

formation of functional NMDAR ion channels, the specific pharmacological and biophysical properties of the heteromeric ion channel are determined by the complement of NR2 or NR3 [21, 22]. This receptor is involved in a wide variety of processes in the central nervous system including synaptogenesis, synaptic plasticity, memory and learning. NMDAR has been established as a crucial molecular switch for synaptic plasticity. The role of NMDAR dependent synaptic plasticity in learning and memory has been explored using both second generation (region specific) and third generation (region specific and inducible) gene knockout techniques. These experiments demonstrate that the CA1-hippocampal NMDAR, a major cellular coincidence detector, is required for formation of hippocampus dependent spatial and non-spatial memories [23]. Additionally, NMDAR has been implicated in excitotoxicity, schizophrenia, various neurodegenerative disorders and aging [24, 25]. Thus, a greater understanding of the modulation of this receptor is likely to be important to the understanding of the physiology and pathophysiology of these processes.

We thought that changes of NMDA receptor density may one of the mechanisms of INH-induced neurologic disorders such as poor concentration and subtle reduction in memory. Also, possible molecular mechanism of these receptor changes may be due to increase of oxidative stress in hippocampus. The results of the present study showed that INH caused a significant increase in MDA, end product of lipid peroxidation, in addition, the treatment with erdostein before the administration of INH led to a significant decrease in lipid peroxidation. INH decreased the level of hippocampal NR2A protein; in addition, erdosteine prevented this decrement.

Recently, it is claimed that INH and/or its metabolites acetylisoniazid, hydrazine and monoacetylhydrazine may cause free radical production in different tissues and an excessive productions build up of reactive oxygen species within the tissue might have led to lipid peroxidation [2]. Their toxic effects can be prevented by N-acetylcysyteine [2]. Various studies showed that, INH exposure led to a marked depletion in the tissue thiol profile. INH depletes the most protective thiols for 2 weeks in rat [2]. The depletion of non-protein thiols is probably due to excessive binding of INH electrophiles generated from INH metabolism to glutathione [2]. Glutathione, a major fraction of non-protein thiols, has strong attraction for electrophiles because of its soft nucleophilic character [2]. The oxidative stress was closely associated with decrease of glutathione levels and alterations in profile of enzymes of GSH levels and alterations in profile of enzymes responsible for disposal of superoxide radicals and hydrogen peroxide. Augmenting cellular antioxidant defense system, especially a precursor of GSH, can be protected against the oxidative damages produced by INH [2]. Previous studies have shown that INH-induced GSH depletion and resulting lipid peroxidation significantly contribute to cellular damage and, these changes were prevented by N-acetylcysteine in the rat liver [2].

Among the possible antioxidant substances used in augmenting the thiol levels, erdosteine seems to be an alternative agent. Erdosteine contains two blocked sulfhydryl groups which are released following its metabolic process. Erdosteine itself does not have free thiole group, but its metabolite has – SH groups [26]. This Erdosteine metabolite (M1) is responsible for free radical scavenging activity through – SH groups [26–28]. Oral erdosteine is immediately converted to the active metabolite (M1, N-thioglycolylhomocysteine) which is free radical scavenging, and mucolytic properties [12]. Therefore, we thought that combination of INH and erdosteine is an appropriate combination because of mucolytic properties of erdostein. The neuroprotective effect of erdosteine could be due to its capability to scavenge INH electrophiles or by repleshing the GSH used during detoxification of these metabolites [26].

Memory and learning are impaired in individuals with age-related neurodegenerative diseases; this is believed to be, in part, a result of excessive generation of reactive oxygen species. The brain is particularly vulnerable to oxidative stress due to a relatively high rate of oxygen radical generation, the presence of high concentrations of easily oxidizable lipids and a relatively deficient antioxidative defense system [29]. Therefore, it can be concluded that INH treatment was toxic to NR2A receptors in hippocampus and this toxicity was probably related to the reactive oxygen species. Erdosteine may have neuro-protective effects on INH-induced neurotoxicity. Further study is necessary to determine whether erdosteine effects on INH-induced neurotoxicity is dose dependent.

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