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Microsomal Lipid Peroxidation, Antioxidant Enzyme Activities in Brain of Male Rats during Long-Term Exposure to Isoniazid

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Abstract

Isoniazid, a broad-spectrum antibiotic employed clinically in the treatment of bacterial infections, is known to cause a number of biochemical dysfunctions and suspected to induce memory decline with age to animals and humans. The present study therefore evaluates the oxidative damage in brain albino male rats. Isoniazid was administered orally at the dose regimen level of 5.0 mg/kg body weight per day in three equal divided doses of 10, 20, 30mg/kg (12 h interval) for 60 days. Control rats were given distilled water for the same period. Administration of Isoniazid significantly decreased the activity of superoxide dismutase. The activity of gamma-glutamyltranspeptidase, the activity of alanine-amino transferase (ALT) and alkaline phosphatase (ALP) as well as the formation of malondialdehyde (MDA) increased ($p<0.05$) compared to the corresponding control. The toxic effects of Isoniazid to the brain antioxidant defence systems were significantly increased. Collectively, the results suggest that therapeutic dose of Isoniazid elicits brain lesions in male rats through induction of oxidative damage and via the inhibitory effect on protein synthesis.

Keywords: Isoniazid; Antioxidant enzymes; Brain lesions; Sperm morphology; Oxidative damage; Rat

Introduction

Isoniazid (Laniazid,Nydrazid) chemically known as isonicotinyl hdrazine (INH) or isotonic acid hydrazide is a broad-spectrum antibiotic employed in the treatment of bacterial infections such as anti-tuberculosis medication in prevention and treatment of infections caused by *Mycobacterium tuberculosis.* It is also most commonly used as antidepressant. Isoniazid is never used on its own to treat active tuberculosis because resistance quickly develops. The emergence of bacteria resistance has limited its use. In spite of this, however, Isoniazid among other counterpart drugs was prescribed either as a new medication or as a continued medication in form of tablets, syrups and injectables or orals. Moreover, the drug has gained wide acceptance in the third world countries because of its easy accessibility, availability and cost effectiveness. As such, the drug can be abused due to self-prescription, which is rife in this part of the world. Isoniazid is pro-drug and must be activated by a bacterial catalase-peroxidase enzyme called KatG [1]. A number of biochemical dysfunctions have been associated with administration of Isoniazid. Isoniazid-induced hepatotoxicity characteristically causes fatty infiltration of the liver [2,3] and liver parenchymal cell damage [4]. Studies have also reported that the drug causes adverse conditions which include rash, abnormal liver functions, hepatitis, anaemia, high anion gap acidosis, peripheral neuropathy, mild central nervous system (CNS) effects. Peripheral neuropathy and CNS effects are associated with the use of Isoniazid and are due to pyridoxine (vitamin B6) depletion. The mechanisms by which Isoniazid elicits these effects are not well understood. However, reactive oxygen species (ROS) has been implicated. Isoniazid caused significant oxidative stress in the liver of rats by reducing the levels of antioxidant enzymes and glutathione (GSH), and elevating the levels of lipid peroxide formed [3]. Immuno histochemical expression of oxidative stress-related markers such as 8-hydroxy deoxyguanosine, heme oxygenase-1 and superoxide dismutase (SOD) has been detected in the livers of rats treated with xenobiotics [5]. Recently, it has been observed that the oxidative damage to testicular cells induced by various xenobiotics, products of abnormal metabolism or ROS can result in testicular dysfunction leading to male infertility [6,7]. Oxidative stress occurs when the production of ROS exceeds the body's natural antioxidant defence mechanisms, causing damage to macromolecules such as DNA, proteins and lipids [8]. To counteract the destructive effects of ROS, cells are equipped with antioxidant defence mechanisms consisting mainly of antioxidant enzymes such as superoxide dismutase, catalase (CAT), GSH peroxidase and small molecular antioxidants like GSH, ascorbic acid and a-tocopherol [9]. Vitamin C (Vit C) and N-acetylcysteine (NAC) are known antioxidants which can scavenge ROS, thus preventing tissue damage. These antioxidants, NAC and ascorbate have been found to be effective in preserving spermatogenesis in an animal model [10] as well as in the medical treatment of male factor infertility [11].

Some drugs have been suspected to cause neurological disorders in animals and humans based on the observation of US military dogs and humans who served during the Vietnam War [12]. To date, exposure to therapeutic drugs or xenobiotics may induce lesions of the brain (neurotoxicity). Thus, the aim of the present study was (a) to evaluate whether therapeutic dose of Isoniazid induces brain lesions and in rats and (b) to investigate the possible toxicological effects of Isoniazid on

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the oxidative stress-markers of the brain so as to provide the possible preventive or curative measures.

Materials and Methods

Chemicals

Isoniazid, Alkaline phosphatise (ALP),Alanine amino transferase (ALT) kits were obtained from Randox Laboratories Ltd, United Kingdom while Epinephrine, glutamyl-3-carboxyl-4-nitroanilide,glycylglycine and thiobarbituric acid (TBA) were purchased from Sigma (St Louis, MO, USA). All other reagents were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK).

Animal treatment

Twenty-eight adult male wistar rats weighing approximately 200–230 g obtained from the Department of Biochemistry, Bells University of science and technology Sango Ota, Nigeria were randomly assigned into 4 groups of 7 animals per group. They were housed in a plastic suspended cage placed in a well ventilated rat house, provided rat pellets and water adlibitum, and subjected to a natural photoperiod of 12 h light and 12 h dark cycle. Rats were given Isoniazid of 5 mg/kg body weight. Rats in Group I served as control and were administered normal saline. Animals in Groups II, III and IV received 10mg/kg, 20mg/kg and 30mg/kg respectively per day over 60 days by oral administration. A period of ten days was allowed for the animals to acclimatize before the drug administration. The experiment lasted for 60 consecutive days.

Necropsy

The animals were fasted overnight and sacrificed by decapitation 24 h after the last treatment and blood was collected by cardiac puncture. Brain tissues were removed and cleared of adhering tissues, washed in ice cold 1.15% potassium chloride and dried with blotting paper. The blood was allowed to clot and centrifuged at low speed (3000g) at room temperature for 15 min. The supernatant was removed and used for the determination of microsomal enzymes.

Enzyme assay

The brain tissues were homogenized in 50mM Tris–HCl buffer (pH 7.4) containing 1.15% KCl and the homogenate was centrifuged at 10,000g for 15 min at 4°C. The supernatant was collected for the estimation of SOD activity by measuring the inhibition of autoxidation of epinephrine at pH 10.2 at 30°C according to Misra [13]. Gamma glutamyl transferase (g-GT) was assayed in the supernatant using L-g-glutamyl-3-carboxyl-4-nitroanilide and glycylglycine as substrates [14]. Protein concentration was determined by the method of Lowry et al. [15].

Lipid peroxidation assay

Lipid peroxidation in post mitochondrial fractions of brain tissues was estimated spectrophotometrically at 532nm by thiobarbituric acid reactive substances (TBARS) and was quantified as malondialdehyde (MDA) according to the method [16] and expressed as mmol/g tissue.

Statistical analysis

The results of the replicates were pooled and expressed as mean ± standard deviation. Student t-test, one way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out. Significance was accepted at $p \leq 0.05$ [17].

Results

Antioxidant and markers enzymes

In order to explore the possibility that isoniazid interferes with antioxidant defence system and thereby induces oxidative damage to brain, the antioxidant levels, brain marker enzymes and marker of oxidative stress was evaluated. The activity of SOD in the post-mitochondrial fraction of rat brain decreased significantly by 92.06%, 39.68% and 26.98% respectively in the animals treated with different doses of isoniazid compared with the corresponding group of control animals (Figure 1). Administration of isoniazid caused a significant increase ($P<0.05$) in the activity of g-GT, a brain marker enzyme, by 165.96%, 165.96% and 261.68% respectively when compared with the corresponding group of control animals (Figure 2).

Marker of oxidative damage

The levels of MDA, a maker of lipid peroxidation, in the brain increased significantly ($P<0.001$) in rats treated with isoniazid by 150%, 200% and 216.67% respectively (Figure 4). Brain total protein decreased significantly by 155.11%, 194.62% and 215.86% respectively, in the isoniazid-treated animals relative to the controls (Figure 3).

Discussion

Although isoniazid has been shown to induce a number of biochemical disorders [18,19] and suspected to cause memory decline with age, evident in human degenerative diseases such as Alzheimer's disease which is accompanied by accumulation of oxidative damage [20]. Our study appears to be the first reporting the ability of the drug to

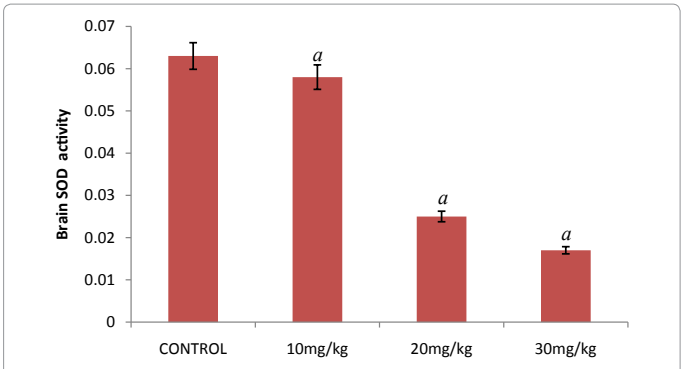

Figure 1: Effects of Izoniacid on rat brain superoxide dismutase (SOD) Values are mean ± SD brain per group. ($^{a}p\leq0.05$) against control, n=7.

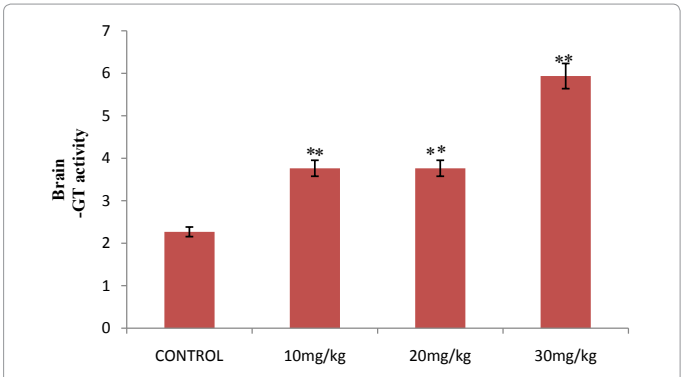

Figure 2: Effects of Izoniacid on rat brain gamma glutamate transferase (ϒ-GT). Values are mean ± SD, 7 per group. (**p≤0.05) against the control, n=7.

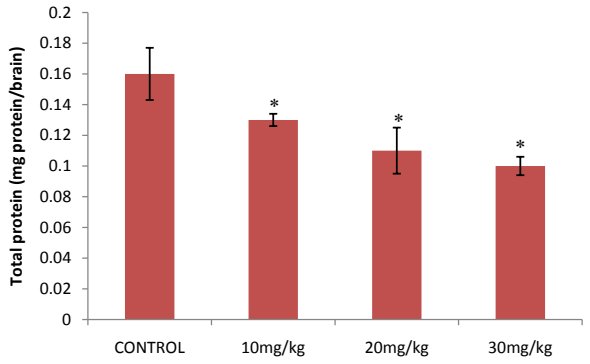

Figure 3: Effects of Izoniazid on rat brain total protein. Values are mean ± SD, (*p≤0.05) against the control n=7.

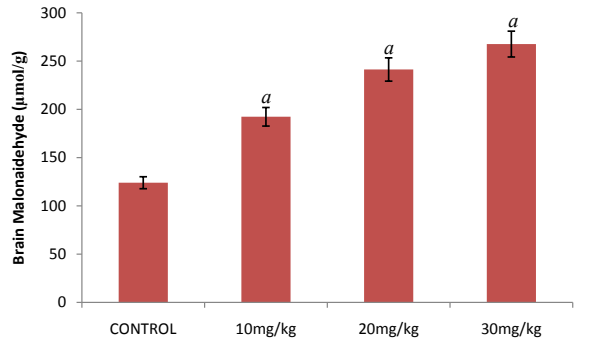

Figure 4: Induced lipid peroxidation in brain tissues by Izoniacid. TBARS (Thiobarbituric acid reactive species) produced were measured at 532nm and the absorbance was compared with that of standard using brain MDA (Malondiadehyde). (^{a}p≤0.05) against the control, n=7.

cause impairment of the brain. In the present study, administration of the isoniazid-treated rats showed decrease in the total protein content of their brain tissue supernatant. This in part shows that decrease in protein content in rats treated with isoniazid may not be due to oxidative stress, but through the direct inhibitory effect of isoniazid on protein synthesis [21] or by cell necrosis. Increase in g-GT activity is characteristic of brain atrophy associated with damage to neuronal cells by many xenobiotics [22,23]. Thus, increase in the activity of g-GT mediated by isoniazid suggests that the antibiotic could mediate toxicity through disruption of normal neurotransmission and transduction or coordination of signals.

The administration of isoniazid tablet suggests decrease in the antioxidant defence molecules. This led to a concomitant increase in the level of MDA, a maker of lipid peroxidation, in brain. The inactivation of the antioxidant enzymes may be caused by excess ROS generated in the system. SOD generally dismutase the superoxide anion radicals into H_2O_2, which is readily degraded by CAT and GSH peroxidase using reduced GSH. Reactive oxygen metabolites such as singlet oxygen, hydroxyl radicals, superoxide and H_2O_2 are known to be cytotoxic agents because of their ability to induce lipid peroxidation in tissue and membranes. In the biological system, the antioxidant enzymes CAT and peroxidase protect against SOD inactivation by H_2O_2, while the SOD reciprocally protects CAT and peroxidises against inhibition by superoxide anion. Our data demonstrate that administration of isoniazid to rats decreased the activity of SOD making superoxide radical (O^{2-}) to be precipitated and further suggests that isoniazid could cause impairment of brain tissues through induction of oxidative stress.

Collectively, the present study reveals that administration of therapeutic dose of Isoniazid to male rats induced brain lesions by disrupting the nervous system, reducing brain antioxidant defence systems and concomitantly increased brain lipid peroxidation. The ability of this Isoniazid-induced toxicity suggests that the antibiotic could mediate brain damage (neurotoxicity) through other mechanisms .However, in view of the importance of this drug in clinical practice; the relevance of our study to human's merits further investigation on the mechanisms by which protective agents prevent Isoniazid-iduced brain toxicity.

Conflict of Interes

The authors declare that there are no conflicts of interest and that the authors of this manuscript have no financial or personal relationship with any organization which could influence the work.

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