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Original Contribution

INVESTIGATION ON BLOOD ECOLOGICAL OXIDATIVE BALANCE IN RATS AFTER TREATMENT WITH RIMIFON

N. Georgieva*

Department of Pharmacology, Animal Physiology and Physiological Chemistry,
Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

The steady-state levels of reactive oxygen species (ROS) determined by the balance between their rates of production and rates of clearance by scavenging mechanisms could be called ecological oxidative balance (EOB) in the biological system. A first-line drug with superior efficacy, used in treating *Mycobacterium tuberculosis* infection, is rimifon (isonicotinic acid hydrazide, INH). One of the major obstacles in the use of INH as antitubercular drug is related to its toxic side effects. The aim of the present study was to determine whether rimifon (INH) disrupts the EOB. For this purpose we investigated the concentrations of malondialdehyde (MDA), nitric oxide (NO) and level of the activities of antioxidant defense enzymes SOD and CAT in blood of albino Wistar rats treated with INH p.o. at 75 mg/kg and 150 mg/kg. Our results confirmed that rats treated with INH had a high blood plasma formation of lipid peroxidation products and that INH treatment was accompanied by increased levels of NO compared to controls ($p<0.001$). The results of the present study showed statistically significant decreased erythrocyte SOD and CAT activities in INH-treated rats as compared to the control healthy untreated ones 30 days after p.o. treatment with INH at doses 75 mg/kg and 150 mg/kg ($p<0.05$). Our results suggested increased oxidative stress and disruption of EOB in rats treated 30 days with INH p.o. 75 and 150 mg/kg.

Key words: Rimifon, INH, NO, MDA, antioxidant enzymes, oxidative stress, ecological oxidative balance

INTRODUCTION

Isonicotinic acid hydrazide (INH, rimifon, isoniazid) is a drug, widely used in chemotherapy of tuberculosis. The preparation is also used for prophylactic treatment of HIV-positive patients that are at high risk to becoming infected with tuberculosis (1-3). Despite its strong antibacterial effect, in some instances rimifon is found to be unsuccessful due to its side toxic effects, including hepatotoxicity (4, 5) and the rapid onset of drug resistance in mycobacteria (6, 7). The toxicity of rimifon is related to the production of free radicals in the course of its metabolic conversion (5, 8, 9) and oxidative stress produced after its administration (10, 11). The mechanism of action of isoniazid is not completely understood and there is still not an acceptable explanation of the extreme sensitivity of *Mycobacterium tuberculosis* to it (12, 13). According to literature data, the bacteriostatic effect of IHN with respect to *Mycobacterium tuberculosis* is mediated by its specific inhibition of mycolic acid synthesis (14, 15). Additional data have linked the bacteriostatic activity of INH against *Mycobacterium tuberculosis* with reactive oxygen species (ROS) formation during its intracellular metabolism (9, 16, 17). Under the influence of endogenous superoxide, INH is activated and this activation brings the formation of other ROS (18, 19). When ROS production exceeds the scavenging capacity of systemic endogenous antioxidant defence, oxidative stress is generated. In a state of oxidative stress and impaired ecological oxidative balance (EOB) biological organisms are not protected from ROS toxic effects (20),

***Correspondence to:** *Assoc. Prof. N. V. Georgieva, PhD, Department of Pharmacology, Animal Physiology and Physiological Chemistry, Faculty of Veterinary Medicine, Trakia University, Students Campus, 6000 Stara Zagora, Bulgaria, E-mail: nvgeorgieva@vmf.uni-sz.bg*

and thus are prone to cell damage, pathologies and diseases. Lipid peroxidation is one of the best markers for the extent of ROS-induced systemic damage (21). Nitric oxide (NO) concentrations could also be useful markers for the appearance and development of pathological alterations and diseases (11, 22-24).

In previous studies of ours, we have reported statistically significantly increased malondialdehyde (MDA) concentrations and reduced activities of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) in liver homogenates of mice with experimentally induced acute (25) and chronic (26) INH toxicity.

The present investigation aimed to evaluate the effect of rimifon upon the blood ecological oxidative balance in Wistar rats after 30-day treatment at doses of 75 and 150 mg/kg, by assaying blood concentrations of MDA, NO and activities of antioxidant enzymes SOD and CAT.

MATERIAL AND METHODS

1. Experimental animals

Trials were carried out with 30 clinically healthy Wistar albino rats weighing 150-200 g, divided into 3 groups of 10 rats each. Chronic experimental INH toxicity was induced by oral treatment of rats with Rimifon – a trade mark of Bristol-Myers Squibb Co. (Connecticut, USA) for 30 days. The first group consisted of healthy untreated rats (controls), animals from the second were treated orally with INH at 75 mg/kg, and from the third – at 150 mg/kg. All treatments were carried out by routine methods approved by toxicological screening and described in the literature (27).

2. Biochemical investigations

Blood samples have been taken the jugular vein. Ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant.

Peripheral blood processing

Collected blood was centrifuged at 3000 g for 15 min and plasma was separated. Then, the plasma was deproteinated with 25% trichloroacetic acid by continuous mixing for 5 min and centrifuged at 2000 g for 15 min.

Erythrocyte processing

The erythrocyte pellet was washed thrice with saline, and the cell suspension was diluted with cold water to lyse the erythrocytes. To 0.2 mL lysate, 1.8 mL water and ethanol/chloroform (3:5/v:v) were then added to precipitate haemoglobin. The tubes were shaken vigorously for 5 min and centrifuged at 2500 g for 20 min. The supernatants were used for determine enzyme activity.

2. 1. Determination of the end products of lipid peroxidation

The deproteinized plasma was used for determining lipid peroxidation products spectrophotometrically using the thiobarbituric acid reactive substance (TBARS) method, and measurement of MDA at 532 nm (28). Results were expressed in μM.

2. 2. Determination of NO

Nitric oxide was assay as nitrates after their reduction to nitrites. Nitrites react with the Griess reagent to form a red azo compound that is quantitated at $\lambda = 540$ nm (29).

2. 3. Determination of superoxide dismutase activity

Erythrocyte lysates were assayed for CuZn-SOD activity as described by Sun et al. (30) with minor modifications. Briefly, the xanthine /xanthine oxidase system was used to generate the superoxide anion $(O_2^{\bullet -})_x$. This anion reduced nitroblue tetrazolium (NBT) to formazan, which was monitored at 560 nm. SOD in the sample removes the $(O_2^{\bullet -})_x$ and inhibits the reduction. The level of this reduction is used as a measure of SOD activity. One unit of enzymatic activity is defined as the amount of enzyme causing 50% inhibition of the reduction of NBT to formazan. Results were expressed as units per gram haemoglobin (U/gHb).

2. 4. Determination of catalase activity

Catalase activity was assessed in the erythrocyte lysates by the method described by Beers and Sizer (31). Hydrogen peroxide was used as a substrate and the decrease in H_2O_2 concentration at 22 °C in phosphate buffer (pH = 7) was followed spectroscopically at 240 nm. One unit of CAT activity is defined as the amount of enzyme that degrades 1μM H_2O_2 per min. Results are presented as units per gram haemoglobin (U/gHb).

2. 5. Haemoglobin concentrations

Haemoglobin concentrations of lysates were analysed by the cyanmethaemoglobin method (32).

2. 6. Statistical analysis

The data were statistically processed by two-way analysis of variance (ANOVA). All results are presented as mean ± SEM. The differences were considered as significant when P values were less than 0.05.

RESULTS

Figures 1 and 2 present blood MDA and NO concentrations, and **Figures 3 and 4** – the blood activities of antioxidant enzymes SOD and CAT in the three groups of Wistar rats. A statistically significant increase in blood MDA levels has occurred in treated as compared to untreated rats: 2.41 μmol/L in the group with INH p.o. 75 mg/kg and 2.69 μmol/L after treatment with INH p.o. at 150 mg/kg, vs 1.53 μmol/L in untreated animals (**Fig. 1**, $p<0.01$).

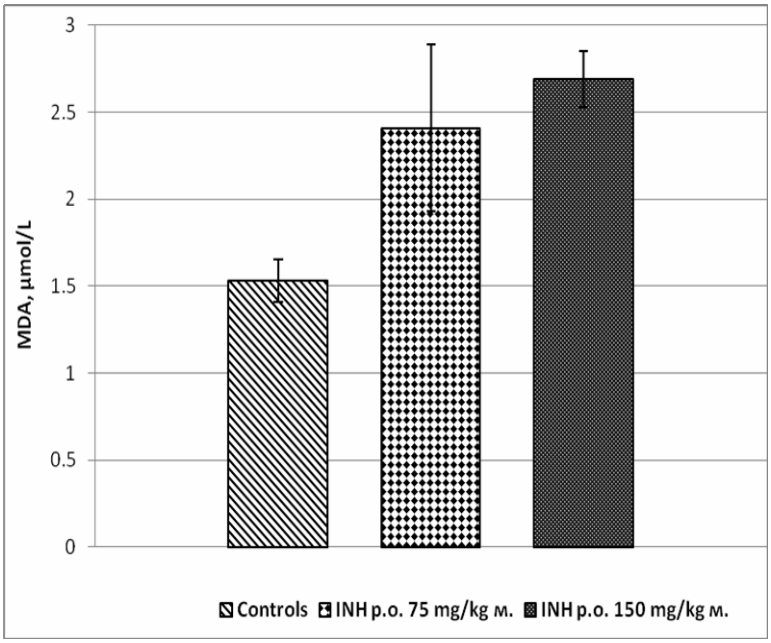

Fig. 1. Concentrations of MDA in plasma of experimental rats.

Blood NO levels were also significantly higher in treated rats than in untreated controls (4.22 μmol/L in the INH p.o. 75 mg/kg group and 9.00 μmol/L in the INH p.o. 150 mg/kg group vs 2.17 μmol/L in controls, **Fig. 2**, $p<0.001$).

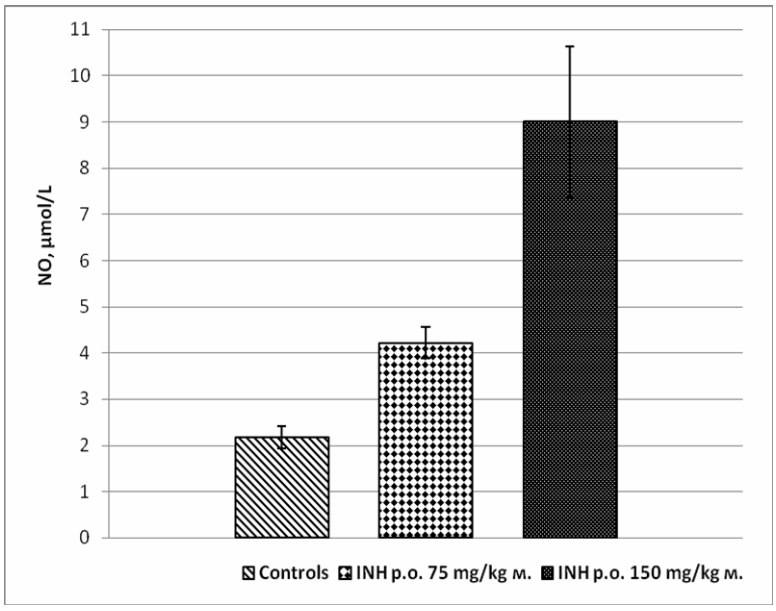

Fig. 2. Concentrations of NO in plasma of experimental rats

According to our data, the 30-day oral treatment with INH at 75 mg/kg and 150 mg/kg reduced the activity of blood antioxidant enzyme SOD (2860.2 and 2607.9 U/mg Hb for the groups that received INH at 75 and 150 mg/kg, respectively vs 4590.6 U/mgHb in control rats, **Fig. 3**, $p< 0.05$).

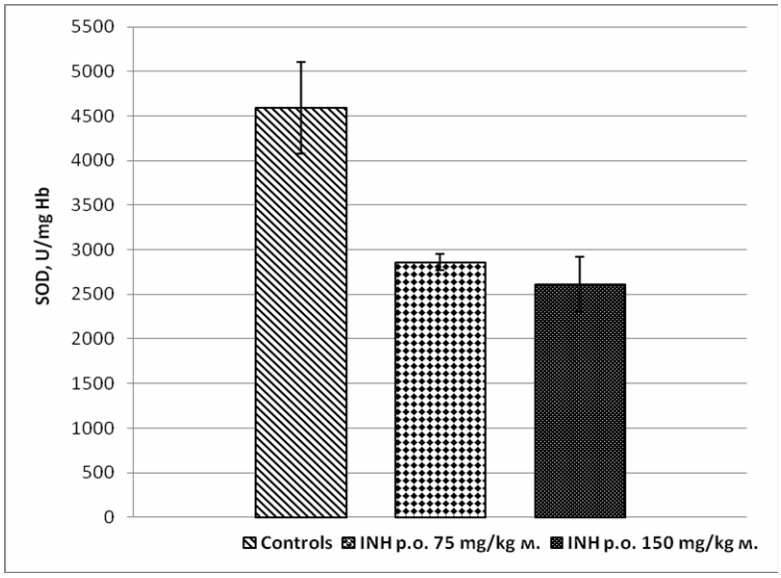

Fig. 3. Levels of erythrocyte SOD activity in experimental rats

The activity of the antioxidant enzyme CAT in INH-treated rats was also reduced (1222.2 U/mg Hb for the group with INH p.o. 75 mg/kg, 1247.0 U/mg Hb for the group with INH p.o. 150 mg/kg, and 2804.8 U/mg Hb in untreated group, **Fig. 4**, $p<0.05$).

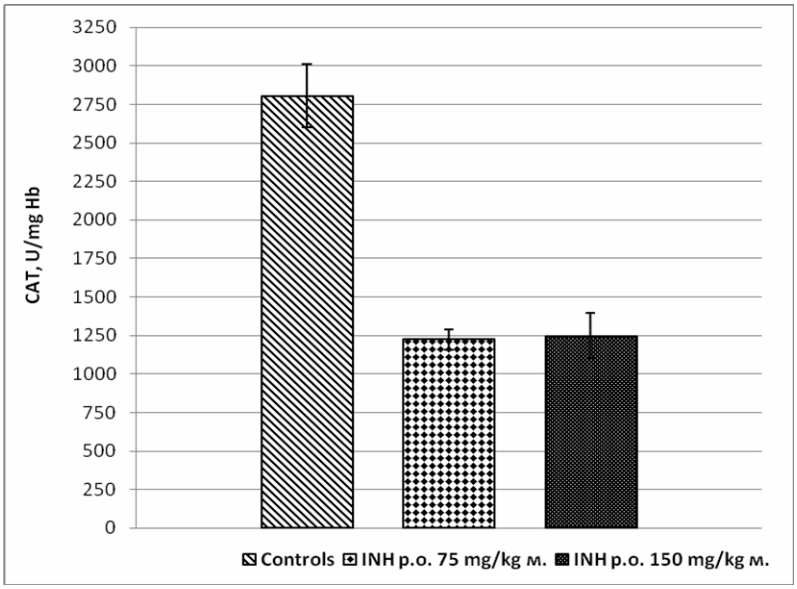

Fig. 4. Levels of erythrocyte CAT activity in experimental rats

DISCUSSION

Lipids are most readily oxidizable and therefore, are most vulnerable to free radical damage via lipid peroxidation. Lipid peroxidation represents a chain of radical oxidative reactions of polyunsaturated fatty acids from membrane phospholipids. These events result in phospholipid degradation, membrane damage and formation of end aldehyde products. One of the end products of lipid peroxidation – MDA, is isolated in urine, blood and tissue and is utilized as a biomarker of radical-induced damage (21). The concentrations of nitric oxide (NO) are useful

markers for onset and development of pathological alterations and disease (23, 33). During the last years, the role of NO in various illnesses is extensively investigated but resulting data are rather contradictory. From one side, NO is considered to be involved in bacterial growth promotion and from the other – to be an important factor in the natural systemic defense against diseases (34). The increased NO production is interpreted as promoter of various diseases as cancer, heart stroke, seizures, diabetes, sepsis etc. (35-38). No concentrations could be useful markers for the onset of inflammations, pathological alterations and disease (11). The higher blood MDA **(Fig. 1)** and No concentrations **(Fig. 2)** in this study after 30-day oral treatment of rats with rimifon could be attributed to enhanced toxic ROS generation resulting from rimifon metabolic conversion on one side (18), as well as to activation of lipid peroxidation (9) and other pathological changes in treated subjects, from the other.

Cells possess a complex and precise antioxidant defense system that prevents ROS formation and/or limit their toxic effect. Superoxide dismutase is an antioxidant enzyme that catalyzes the conversion of the highly reactive superoxide anion radical $(O_2^{\bullet-})_x$ – a chain-reaction promoter, to less reactive species. This is the first step of systemic antioxidant defense (39). The antioxidant enzyme CAT protects cells from drug-induced consumption of oxygen via degradation of hydrogen peroxide or by direct interaction with the drug and thus, is essential for cells' adaptation to oxidative stress (40). Under normal physiological conditions, there is an ecological oxidative balance (EOB) between free radical generation and the scavenging capacity of systemic antioxidant defense (20). In the EOB state, aerobic organisms are maximally protected against the toxic ROS influence (41).

The increased MDA **(Fig. 1)** and NO **(Fig. 2)** and lower blood activities of SOD **(Fig. 3)** and CAT **(Fig. 4)** in rats treated orally for 30 days with INH provided evidence for reduced endogenous enzyme antioxidant defense i.e. impaired antioxidant status. This disturbance is probably related to produced oxidative stress and impaired EOB in favour of the prooxidants in the organism of treated rats resulting probably from abnormal ROS production during rimifon metabolic conversion. In a state of oxidative stress and impaired EOB, biological systems are most vulnerable to the toxic oxidative ROS effects (20). Oxidative toxic effects of rimifon, including its hepatotoxicity could be attributed to impaired EOB and oxidative stress produced during its metabolism.

The results from the present experiments suggested that impaired ecological oxidative balance could be a possible reason for the systemic oxidative toxic effects of rimifon.

CONCLUSIONS

Rimifon produces oxidative stress and impairs systemic EOB in treated rats in favour of prooxidants, most probably following abnormal ROS production during its metabolic conversion. The impaired EOB after experimentally provoked INH toxicity could be a reason for the oxidative toxic effects of INH.

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