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Effects of Subacute Treatment of Thiourea on Antioxidant and Immunopotential Marker Enzymes and Lipid Peroxidation of Rats: A Drinking Water Study

ISMAIL CELIK*, HUSEYIN SUZEK† and YASIN TULUCE
Department of Biology, Science and Art Faculty, Yuzuncu Yil University, Van 650 080, Turkey
Fax: (90)(432)2251114; Tel: (90)(432)2251083-2278
E-mail: icelik65@yahoo.com

The effect of subacute 25 days treatment of thiourea, an inhibitor of diene conjugate formation as hydroxyl radical scavengers were tested as terminators of lipid peroxidation (malondialdehyde = MDA), antioxidant [reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT] and immunopotential [adenosine deaminase (ADA) and myeloperoxidase (MPO) defense systems marker enzymes in the liver tissue of rats under laboratory conditions. 250 ppm of thiourea was administered orally to 8 rats *ad libitum* during the tests for 25 days consecutively. Malondialdehyde content, antioxidant and immunopotential marker enzyme of liver were determined after treatment. According to results, while the treatment of thiourea caused significant decrease in MDA content, GSH level, GR activity and immunopotentical enzyme activities decreased in comparison to control rats. CAT, GPx, SOD and GST activities did not change significantly, These results suggest that thiourea may not only reduce oxidative damage by induced oxygen reactive radicals in animals, but affect immunopotential enzymes too.

Key Words: Thiourea, Malondialdehyde, Antioxidant and Immunopotential defense enzymes, Rats.

INTRODUCTION

Thiourea and its metabolites are found in a wide variety of biologically active compounds. Examples are: diafenthurion, which is used as insecticide[1]; methimazole which is used in the treatment of Graves' disease[2]; ethylenethiourea, a breakdown product and metabolite of ethylene bis(dithiocarbamate) fungicides like maneb and zineb[3, 4] and a listed carciogen, is of environmental interest.

There is abundant evidence that the effects of thiourea and its metabolities have been investigated up to date. Flavin containing monooxygenases are mostly responsible for oxidation resulting in the formation of sulfonic acid, that thiocarbamide is generally accepted to be oxidation of thiosulfur[5, 6]. Methimazole,

†Department of Biochemistry, Health High School, Mugla University, Mugla, Turkey.

which is the metabolite of thiourea, has shown induction of cytochrome P450 isozymes of the 2B and 1A classes. Phenobarbital and β-naphthoflavone, which are the derivatives of thiourea, increase the oxidative metabolism[7]. On the other hand, thiourea and dimethhylthiourea also are highly permanent molecules that are also effective scavengers of H_2O_2, OH and hypochlorous acid *in vitro*[8–10] and are being widely used to prevent O_2 metabolite-induced injury to lung, kidney and other tissues[11–14]. Pretreatment of mitochondria with deferoxamine (DFO) and dimethyl thiourea (DMTU) prevented OH production and markedly reduced lipid peroxidation without appreciably altering iron release caused by H_2O_2. Simultaneous treatment of either DFO or DMTU with H_2O_2 significantly reduced lipid peroxidation and also prevented OH production without causing marked decrease in iron release[15]. On the other hand, treatment of rats with DMTU, metabolite of thiocarbamide, just before lipopolysaccharide injection, resulted in a decreased lipid peroxidation and an increase in phosphatidylcholine biosynthesis, although it did not prevent body weight loss or increase in lung weight and lung protein content associated with the lung injury induced by lipopolysaccharide[16].

In this study, our attention is focussed on chemopreventive and anti-oxidant activity of the commonly used thiourea, since these properties are closely related to free radicals prevention and therapy of health. In order to achieve a more rational design of thiourea, it is necessary to clarify the mechanisms of thiourea improve effects comprehensively. For this aim, the treatment of thiourea was done orally because the effect of medicine represents a well characterized *in vivo* effect model system. The liver was chosen due to its important role during detoxification in degradation and bioactivation of thiourea.

EXPERIMENTAL

All chemicals used in this study were supplied by Sigma Chemical Co. (St. Lovis, USA). For antioxidant enzymes analysis kits were supplied by Randox Laboratories Ltd. Rats (Sprague-Dawley albino) weighing 150–200 g were provided by the animal house of the Medical School of Yüzüncü Yil University and were housed in 2 groups, each group containing 8 rats. All animals were fed a group wheat-soybean-meal-based diet and water *ad libitum* in stainless cages, and received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals'. The animals were housed at 20 ± 2°C an in daily light/dark cycle.

Rat treatment: Rats were exposed to thiourea (dissolved in tap water) for twenty-five days by oral administration of 250 ppm dosage *ad libitum* during the tests consecutively. Control rats were given only drinking tap water. Daily water consumption of rats was *ca.* 30 ± 5 mL during the tests.

At the end of the treatment, rats were anesthetizaed by inhalation of diethyl ether and they were then sacrificed. The livers and muscles were dissected and put in petridishes. After washing the tissues with physiological saline (0.9% NaCl), samples were taken and kept at –87°C until analysis. The tissues were

homogenized for 5 min in 0.115 M KCl solution (1 : 5 w/v) using a glass-porcelain homogenizer (20 kHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000 × g for 15 min. All processes were carried out at 4°C. Supernatants were used to determine MDA.

Biochemical analysis: The liver MDA concentration was determined using the method described by Jain *et al.*[17] based on thiobarbituric acid (TBA) reactivity. Briefly, 0.2 mL supernatant obtained from tissues, 0.8 mL phosphate buffer (pH 7.4), 0.025 mL butylated hydroxytoluene (BHT) and 0.5 mL 30% trichloroacetic acid (TCA) were added to the tubes and mixed. After 2 h incubation at –20°C, the mixture was centrifuged (400 g) for 15 min. After this, 1 mL supernatant was taken and added to each tube and then 0.075 mL of 0.1 mol ethylenediaminetetraacetic acid (EDTA) and 0.25 mL of 1% TBA were added. These tubes with teflon-lined screw caps were incubated at 90°C in a water bath for 15 min and cooled to room temperature. The optical density was measured at 532 for tissues MDA concentration (Novaspec II Pharmacia-Biotech, Biochrom Ltd., UK).

GSH concentration was measured using the method described by Beutler *et al.*[18] Briefly, 0.2 mL supernatant was added to 1.8 mL distilled water. 3 mL of the precipitating solution (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl in 100 mL distilled water) was mixed with haemolysate. The mixture was allowed to stand for *ca.* 5 min and then filtered. 2 mL of filtrate was taken and added into another tube and then 8 mL of the phosphate solution and 1 mL 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were added. A blank was prepared with 8 mL of phosphate solution, 2 mL diluted precipitating solution (three parts to two parts distilled water) and 1 mL DTNB reagent. A standard solution of the glutathione was also prepared (40 mg/100 mL). The optical density was measured at 412 nm in the spectrophotometer.

CAT activity was determined using the Beutler *et al.*[19] method. Briefly, 1 mL tris-HCl, 5 mM EDTA (pH = 8), 10 mM H_2O_2 and H_2O were mixed and the rate of H_2O_2 consumption at 240 nm and 37°C was used for quantitative determination of CAT activity, GST was assayed at 25°C spectrophotometrically by following the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm as described by Mannervic and Guthenberg[20]. GR activity was assayed at 37°C and 340 nm by following the oxidation of NADPH by oxidized glutathione (GSSG)[19]. The GSSG in the medium was reduced to GSH by GPx and NADPH. The activity of GPx was assayed at 37°C and 340 nm by calculating the difference in absorbance values during the oxidation of NADPH[19]. SOD activity was measured at 505 nm and 37°C and calculated using inhibition percentage of formazan formation[21]. ADA was assayed by the method described by Giusti[22]. ADA assay is based on indirectly measuring the formation of NH_3 produced when ADA acts in excess of adenosine. The release of ammonia was determined colorimetrically at 630 nm after the development of an intense blue colour with

hypochlorite and phenol in an alkaline solution. MPO was assayed bythe Bradley *et al.*[23] method.

Analysis of data: All data were expressed as mean ± standard deviation (SD). For statistical analysis, the SPSS/PC$^+$ package (SPSS/PC$^+$, Chicago, USA) were applied. For all parameters, means and SD were calculated according to the standard methods. Mann Whitney U-test for differences between means of the treatments and the control rats was employed. The significance level was accepted at $p = 0.05$ for all tests.

RESULTS AND DISCUSSION

The treatment of rats with thiourea produced changes in the level of thiourea on various tissues MDA constituents of rats (Table-1). To find out the significance of MDA constituents, GSH level and enzyme activities changes on exposure to TU for 25 days; the data obtained have been subjected to Man Whitney-U test.

According to results, while the treatment of thiourea caused significant decreases in MDA content, GSH and immunopotential enzyme activities, GR activity decreased in comparison to control rats.

TABLE-1
EFFECT OF THIOUREA ON MDA CONTENT, ANTIOXIDANT AND IMMUNOPOTENTIAL MARKER ENZYMES IN THE LIVER OF RAT (Mean ± SD)

Parameters	Control	Thiourea	P
MDA (nmol/g)	91.19 ± 2.41	19.83 ± 6.30*	≤ 0.001
GSH (mg/g)	74.54 ± 7.60	42.51 ± 3.18*	≤ 0.012
ADA (μ/g)	19.38 ± 0.49	14.98 ± 0.66*	≤ 0.012
MPO (μ/g)	174.62 ± 15.19	125.72 ± 21.52*	≤ 0.022
CAT (μ/g)	56.16 ± 9.96	78.17 ± 20.90	≤ 0.060
GR (μ/g)	0.11 ± 0.01	0.07 ± 0.02*	≤ 0.012
GST (μ/g)	30.86 ± 1.33	32.81 ± 3.73	≤ 0.143
GPx (μ/g)	31.90 ± 1.80	33.60 ± 1.10	≤ 0.080
SOD	984.02 ± 0.95	984.79 ± 0.08	≤ 0.998

Each value represents the mean ± SD. *$p \leq 0.05$.

Recent developments in biomedical science have shown that free radicals are involved in many diseases. They attack the unsaturated fatty acids in the biomembrane resulting in membrane lipid peroxidation, which is strongly connected to ageing, carcinogenesis and atherosclerosis. Free radicals also attack DNA and cause mutation leading to cancer. In addition, lipid peroxidation is an important factor of deterioration in the processing and storage of food. Therefore, it is important to search for new effective radical scavengers[24].

In our study, thiourea was preferred because information on its chemopreventive effects on higher animals is very limited as an *in vivo* and oral model and thiourea and its metabolites also are found in a wide variety of biologically active compounds.

So far, no study examining the effect of thiourea *in vivo* has been made on rats' liver MDA content, GSH level, antioxidant and immune defense marker enzymes as a drinking water study model. In addition, due to inconsistent factors like treatment time and manner, purity and species tissue differences etc., it is difficult to compare data provided from different laboratories such as the test chemicals for biological effect.

The results of the present study indicate that thiourea possesses the antioxidant agent properties. This is evidenced from our observation that, upon thiourea treatment of rats *in vivo*, the level of MDA decreased, which may correlate to the decrease of reactive oxygen radicals. The reasons for such effect of thiourea are not understood at present decisively, but it is conceivable that thiourea is an anti-oxidant like other anti-oxidants. It also might decrease hydrogen peroxide (H_2O_2), hydroxyl (OH) and superoxide (O_2) radicals as a result of aerobic conditions in the organisms, leading to an increase in lipid peroxidation. However, cytochrome P_{450} isozymes of the 1A and 3B subclasses have been suggested to be involved in the oxidation of the thiourea moiety of ethylene thiourea and methimazole, since α-naphthyl thiourea and dexomethasone-induced rat liver cytocrome P 450 activity decreased upon preincubation with these two thiourea moieties[7, 25].

In addition to decreased lipid peroxidation, thiourea treatment can affect the activities of protective non-enzymatic, enzymatic antioxidants and immunopotential marker enzymes in organisms. GSH and activities of CAT, GPx, GR, SOD and GST are known to serve as protective responses to eliminate reactive free radicals. In this study, the GSH level and GR activity decreased, but the other antioxidants did not change. The reason for such effects of thiourea is not understood at present, but the decrease of GSH may be due to the result of the decreased activities of enzymes ruling in GSH snythesis. Also, the decreased GR activity may be due to the effects to eliminate reactive free radicals by thiourea. Another reason, thiourea may lead to the inhibition of this enzyme synthesis into cytoplasm as a result of mRNA breakdown or cellular transcription mechanisms. Also, in the present study, thiourea inhibited the activities of ADA and MPO. Inhibition of ADA by thiourea, as well as the inhibition of adenosine transport and metabolism, might decrease the level of adenosine. This decrease may indicate depressed cell-mediated immunity. ADA is essential for the proper functioning of the vertebrates' body immune system. Because ADA is the major enzyme responsbile for the degradation of Ado, the inhibition of its activity should represent one of the best ways to increase the accumulation of Ado in tissues under chemical stress conditions. Also, inhibition of MPO in the liver by thiourea might cause the destruction of immunopotential. Although the treatment, materials of study and the setting of study are different, this result is in accordance with our result and supports the above claims.

We conclude that thiourea treatment in rats and other animals could prevent lipid peroxidation and protect from reactive oxygen radicals, but may cause the destruction of ingested organisms and it is also postulated that liver MDA and anti-oxidant defense system might offer a marker of choice for chemo-prevention of such chemicals.

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