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Tertiary butyl hydroperoxide induced oxidative damage in mice erythrocytes: Protection by taurine

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Abstract

The present study was undertaken to investigate the protective role of taurine, against t-butyl hydroperoxide (TBHP) induced oxidative stress in murine erythrocytes. Erythrocytes were treated either with TBHP alone or with taurine, followed by TBHP exposure. TBHP-induced oxidative stress increased methemoglobin formation, lipid peroxidation and protein carbonylation in erythrocytes. The same exposure, however, depleted cellular GSH content and altered the activities of the antioxidant enzymes as well as of methemoglobin reductase; reduced activities of Ca^+ and Na^+/K^+ ATPase and intracellular ATP levels. Taurine transport inhibitor, β-alanine, treated erythrocytes showed increased phosphatidylserine externalization and ROS formation on TBHP exposure and taurine could not revert the effect. TBHP exposure increased intracellular calcium and upregulated the level of calpain. Administration of taurine could, however, prevent the TBHP induced oxidative imbalance. Electron micrographs of erythrocytes showed changed morphology with an increase in the number of echinocytes. Taurine treatment could restore the normal levels of the antioxidant enzymes and metabolites of the erythrocytes. Results suggest that the oxidative insult introduced in erythrocytes by TBHP administration is prevented by taurine mainly via membrane stabilization.

Keywords: Erythrocytes; TBHP; ROS; Oxidative stress; β-Alanine; Taurine

1. Introduction

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to detoxify the reactive intermediates. Oxidative stress caused by TBHP is well documented. TBHP can be released in air, soil and ground water during its production and from waste water treatment plants. It is toxic in any route of exposure and causes extreme discomfort to gastrointestinal track, eyes and respiratory system [1–5].

Blood is a fluidic connective tissue that helps to carry oxygen and nutrients to all body parts. Being composed of different cell types, blood along with lymphatic system, form a major line of defense against foreign bodies or antigens. When cells or tissues are exposed to external toxin like TBHP, membrane permeability occurs [1,6] at the same time the membrane is hyperpolarized and water pores are formed. Reports [7] also suggest that TBHP selectively inhibits membrane bound enzymes, increases the formation of methemoglobin and causes aggregation of some membrane proteins into high-molecular-weight polymers. In the red cell cytosol TBHP is either reduced by glutathione, Gpx reducing system or by hemoglobin. When hemoglobin reacts with TBHP, t-butoxyl radicals are formed, which then react with membrane lipids to initiate peroxidation [8]. Chain of reactions are initiated which include decomposition of the hydroperoxides to various end products like malondialdehyde [9], alkenals, aldehydes, ketones and hydrocarbon gases [10]. Extensive lipid peroxidation results in membrane disruption and haemolysis [11].

Cellular antioxidants like glutathione and L-ascorbate, to some extent prevent membrane disruption by scavenging

Abbreviations: TBHP, tertiary butyl hydroperoxide; ROS, reactive oxygen species; GSH, glutathione; GSSG, glutathione disulfide; CAT, catalase; GR, glutathione reductase; GST, glutathione-S-transferase; SOD, superoxide dismutase; GPx, glutathione peroxidase; G-6-PD, glucose-6-PO_4 dehydrogenase; MDA, malondialdehyde; MR, methemoglobin reductase; NADPH, nicotinamide adenine dinucleotide phosphate disodium salt; NADP, nicotinamide adenine dinucleotide reduced sodium salt; DNPH, 2,4-dinitrophenylhydrazine; NEM, N-ethylmaleimide; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); ATP, adenosine triphosphate; FACS, fluorescence activated cell sorter; SEM, scanning electron microscopy.

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the t-butoxyl radicals. Various reports suggest the beneficial roles of vitamins C, flavonoids [12,13], against TBHP induced toxicity in human red blood cells. Although taurine is a well-established antioxidant and has been shown to protect various body organs from oxidative stress due to heavy metals [14–22] drugs [23–25] and chemicals [26], little is known about its beneficial role in TBHP induced cytotoxicity in erythrocytes. The aim of the present study was, therefore, to investigate the in vitro protective role of taurine against TBHP induced oxidative damage in mice erythrocytes. Activities of (a) the intracellular enzymes, CAT, GST, GR, G-6-PD, GPx, MR, and Ca^{2+} and Na^{+}/K^{+} ATPase, (b) the levels of ATP, GSH and GSSG, (c) the levels of lipid peroxidation end products and protein carbonylation, (d) the change in intracellular ROS and Ca^{2+}, (e) microscopic analysis of normal and treated erythrocytes and (f) FACS analysis and calpain activation due to oxidative stress were investigated. In addition, the potent taurine transport inhibitor β-alanine [27] was also administered to the mice to evaluate the protective effect of TBHP on erythrocytes in vitro.

2. Materials and methods

2.1. Chemicals

Dulbecco's modified Eagle's medium (DMEM) was purchased from Invitrogen, India. Taurine, β-alanine, TBHP, Bradford reagent, BAPTA-AM and Drabkin's reagent were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Anti-calpain antibody, Indo-1, Annexin V, DCFDA were purchased from Abcam (Cambridge, UK). All other chemicals were bought from Sisco Research Laboratory, India.

2.2. Animals

Adult albino mice of Swiss strain, weighing between 20 and 25 g were purchased from M/S Gosh Enterprises, Kolkata, India. Animals were acclimatized under laboratory condition for two weeks prior to the experiments. All the experiments with animals were carried out according to the approval and guidelines of the Bose Institute animal ethical committee (the permit number is: 95/99/CPCSEA).

2.3. Experimental design

2.3.1. In vitro

Blood was collected in anticoagulant containing vials by puncturing the heart of the six weeks old mice after anasthesia. Erythrocytes were isolated following the method as described elsewhere [28]. The erythrocytes were washed and suspended in DMEM ($\sim 5 \times 10^6$ cells/ml). All experiments were performed with different sets of erythrocyte suspensions. Untreated erythrocyte suspension was set as control.

To determine optimum dose of TBHP in erythrocytes' pathophysiology, the washed erythrocytes were treated with different concentrations of TBHP (1–10 μM) and were incubated at 37 °C for 60 min in a 5% CO_2 atmosphere. Intracellular ROS was estimated spectrofluometrically by incubating washed erythrocytes with 10 μM of the fluorescent dye DCFDA for 30 min at 37 °C in dark.

To determine the optimum time of TBHP exposure, different sets of erythrocytes were exposed with the working dose (5 μM) of TBHP for a time span ranging from 0 to 60 min. The increase in ROS was considered as the parameter to determine the optimum treatment time.

After standardization of the optimum dose and time required for TBHP, further experiments were conducted to determine the dose for taurine. Taurine concentrations tested were from 2 to 20 mM. Erythrocytes not treated with TBHP were considered as normal control and erythrocytes exposed to 5 μM TBHP were treated as toxin control. Taurine (2–20 mM) was added to the cell suspension with toxin and incubated for 60 min.

After determining the dose of taurine administration, taurine was added to erythrocytes and incubated for 30 min (pre-treatment). After incubation, TBHP was added and further kept for 30 min at 37 °C. In another set, taurine and TBHP was simultaneously added to erythrocyte suspension and incubated for 30 min at 37 °C. All the incubations were performed in 5% CO_2 atmosphere.

2.3.2. Taurine depletion studies

For intracellular taurine depletion, mice were fed β-alanine (3% in aqueous medium) for 14 days prior to sacrifice [29]. Erythrocytes were collected from the animals as described before, washed in PBS and used for further treatment with TBHP and taurine. No dose dependent studies were conducted on β-alanine treated erythrocytes. These erythrocytes were exposed to TBHP and taurine and were analyzed for ROS formation and phosphatidylserine externalization only, by flow cytometric analysis.

2.4. Protein estimation

Protein concentrations of experimental samples were determined by the method of Bradford [30].

2.5. Hemolysis and determination of hemoglobin content

Hemolysis was done by freeze thawing the packed erythrocytes three times. The lysate was centrifuged at 5000 × *g* for 10 min at 4 °C to pellet debris and unlysed cells and the clear supernatant or the hemolysate was collected. Hemoglobin content was estimated from the hemolysate using Drabkin's hemoglobin estimation kit (Sigma) following the manufacturer's protocol.