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Propylthiouracil (PTU)-induced hypothyroidism alleviates burn-induced multiple organ injury

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Abstract

Oxidative stress has an important role in the development of multiorgan failure after major burn. This study was designed to determine the possible protective effect of experimental hypothyroidism in hepatic and gastrointestinal injury induced by thermal trauma. Sprague Dawley rats were administered saline or PTU (10 mg kg^{-1} i.p.) for 15 days, and hypothyroidism was confirmed by depressed serum T_3 and T_4 concentrations. Under brief ether anesthesia, shaved dorsum of rats was exposed to 90 °C (burn group) or 25 °C (control group) water bath for 10 s. PTU or saline treatment was repeated at the 12th hour of the burn. Rats were decapitated 24 h after injury and tissue samples from liver, stomach and ileum were taken for the determination of malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and collagen contents. Formation of reactive oxygen species in tissue samples was monitored by using chemiluminescence (CL) technique with luminol and lucigenin probes. Tissues were also examined microscopically. Tumor necrosis factor (TNF)-α and lactate dehydrogenase (LDH) were assayed in serum samples. Severe skin scald injury (30% of total body surface area) caused a significant decrease in GSH level, which was accompanied with significant increases in MDA level, MPO activity, CL levels and collagen content of the studied tissues ($p < 0.05$–0.001). Similarly, serum TNF-α and LDH were elevated in the burn group as compared to control group. On the other hand, PTU treatment reversed all these biochemical indices, as well as histopathological alterations induced by thermal trauma. Our results suggest that PTU-induced hypothyroidism reduces oxidative damage in the hepatic, gastric and ileal tissues probably due to hypometabolism, which is associated with decreased production of reactive oxygen metabolites and enhancement of antioxidant mechanisms.
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Keywords: PTU; Experimental hypothyroidism; GSH; MPO; Lipid peroxidation

1. Introduction

Following severe burn, all tissues are subjected to ischemia and consequently to reperfusion during burn shock resuscitation [1,2], which initiates a series of deleterious events and exacerbates tissue injury that occurred during low flow state [3]. Thermal trauma elicits a systemic inflammatory response, where the generation of reactive oxygen radicals plays an important role, and causes multiple organ failure [4–6]. The inflammatory response to thermal injury is extremely complex, resulting in local tissue damage and deleterious systemic effects in all the organ systems distant from the original wound. Several studies demonstrated that burn is associated with lipid peroxidation, which is an autocatalytic mechanism leading to oxidative destruction of cellular membranes, and their destruction can lead to the

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production of toxic, reactive metabolites and eventually to cell death [3,4].

Thyroid hormones are among the most important humoral factors involved in setting the basal metabolic rate [7]. Since one of the major effects of thyroid hormones is to increase mitochondrial respiration through changes in the number and activity of mitochondrial respiratory chain components [8], thyroid hormones have a considerable impact on oxidative stress [9]. Accordingly, it was reported that thyroid state may alter the changes in oxidant and antioxidant systems [10]. The results of in vivo and in vitro studies point out that high concentrations of thyroid hormones can facilitate the metabolism of oxygen in aerobic conditions and stimulate free radical generation. Hypermetabolism induced by thyrotoxicosis has been shown to aggravate oxidant-mediated tissue injury [11,12]. Otherwise, metabolic depression brought about by hypothyroidism has been associated with a decrease in oxidant production and protects tissues against lipid peroxidation [13,14].

Based on these reports, this study was designed to determine the role of thyroid state in thermal trauma-induced oxidative damage of the liver, stomach and ileum by evaluating the extent of tissue injury through biochemical and histological analyses.

2. Materials and methods

2.1. Animals

Sprague Dawley rats of both genders, weighing 200–250 g, were fasted for 12 h, but were allowed free access to water before experiments. Rats were kept in a room at a constant temperature 22 ± 2 °C with 12 h light and 12 h dark cycles, in individual wire-bottomed cages and fed standard rat chow. All experimental protocols were approved by the Marmara University, School of Medicine, Animal Care and Use Committee.

2.2. Experimental design and thermal injury

Rats were treated intraperitoneally with either 6-*n*-propyl-2-thiouracil (PTU, 10 mg kg^{-1}; Sigma Chemical, St. Louis, MO) or saline (1 ml/kg) for 15 days before the induction of burn. Under brief ether anesthesia, dorsum of the rats was shaved, exposed to 90 °C water bath for 10 s, which resulted in partial-thickness second-degree skin burn involving 30% of the total body surface area. All the animals were then resuscitated with physiological saline solution (10 ml/kg, subcutaneously). PTU or saline treatment was repeated at the 12th hour of the burn injury. Rats in both saline (burn; $n = 8$) and PTU-treated (PTU-burn; $n = 8$) groups were then decapitated at 24th hour after burn injury. In another group of rats, which were treated with either saline (control; $n = 8$) or PTU ($n = 8$), the same protocol was applied except that the dorsums were dipped in a 25 °C water bath for 10 s.

After decapitation, trunk blood was collected and the serum samples were stored for the determination of T_3 and T_4 levels using radioimmunoassay. In PTU-burn group, hypothyroidism was confirmed by depressed serum T_3 and T_4 concentrations. In extra serum samples, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured as indicators of liver function, while lactate dehydrogenase (LDH) and tumor necrosis factor (TNF)-α were assayed for the evaluation of generalized tissue damage. In order to evaluate the presence of oxidant injury, tissue samples from the liver, stomach and kidney samples were stored at −80 °C for the determination of malondialdehyde (MDA) and glutathione levels. Tissue-associated myeloperoxidase (MPO) activity, as an indirect evidence of neutrophil infiltration, and collagen content as a free radical-induced fibrosis marker were also measured.

2.3. Blood assays

Serum AST and ALT [15] concentrations and LDH levels [16] were determined spectrophotometrically using an automated analyzer. Plasma levels of tumor necrosis factor-α (TNF-α), was quantified using enzyme-linked immunosorbent assay (ELISA) kits specific for the previously mentioned rat cytokines according to the manufacturer's instructions and guidelines (Biosource Europe S.A., Nivelles, Belgium). These particular assay kits were selected because of their high degree of sensitivity, specificity, inter- and intra-assay precision, and small amount of plasma sample required to conduct the assay. TNF-α in the serum samples was expressed as pg/ml.

2.4. Malondialdehyde and glutathione assays

Tissue samples were homogenized with ice-cold 150 mM KCl for the determination of malondialdehyde (MDA) and glutathione (GSH) levels. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously [17]. Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and results are expressed as nmol MDA/g tissue. GSH measurements were performed using a modification of the Ellman procedure [18]. Briefly, after centrifugation at 3000 rpm for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l $Na_2HPO_4 \cdot 2H_2O$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of $13{,}600\ M^{-1}\ cm^{-1}$. Results are expressed in μmol GSH/g tissue.

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