Methimazole-induced hypothyroidism in rats ameliorates oxidative injury in experimental colitis

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Abstract
Depression of metabolism by hypothyroidism decreases oxidant production and thus protects tissues against oxidant damage. Moreover, it is well-known that abnormal gut motility is a common manifestation in hypo/hyperthyroidism. In this study, we aimed to investigate the putative beneficial effects of methimazole on oxidative injury and dysmotility in a rat colitis model. Methimazole (0·04%) was administered in drinking water starting 15 days prior to induction of colitis. Colitis was induced by intracolonic administration of trinitrobenzene sulfonic acid (30 mg/ml; 0·8 ml) in ethanol. Six days after the induction of colitis, the fecal output was measured and used as an index for colonic motility. All rats were decapitated on the seventh day. The distal colon was weighed and the mucosal lesions were scored. Colonic lipid peroxidation (LP) and glutathione (GSH) measurements were performed. The macroscopic score, the colonic wet weight and LP values of the euthyroid colitis group were found to be higher than those of the control group (P<0·05–0·001). All these parameters were reduced in the methimazole-treated colitis group (P<0·01–0·001). The decrease in colonic GSH levels in the colitis group was completely abolished in the methimazole-treated colitis rats (P<0·01). Induction of colitis increased the average fecal output compared with the control group (P<0·05) and methimazole in the colitis group exaggerated the fecal output (P<0·001). In conclusion, methimazole reduces colonic oxidative injury probably due to hypometabolism, which is associated with a decrease in the production of reactive oxygen intermediates and an increase in the response of antioxidant systems.


Introduction
There is a good deal of evidence to suggest that thyroid state may alter the induction and course of various inflammatory responses in the body. One of the major effects of thyroid hormone is to increase mitochondrial respiration through changes in the number and activity of mitochondrial respiratory chain components (Roodyn et al. 1965, Nishiki et al. 1978). A hypermetabolic state due to hyperthyroidism has been shown to accelerate reactive oxygen metabolite production in the mitochondria and to induce changes in the antioxidant system of the tissues (Asayama & Kato 1990, Venditti et al. 1997). Hypermetabolism induced by thyrotoxicosis is known to aggravate oxidant-mediated tissue injury. Otherwise, metabolic depression brought about by hypothyroidism has been associated with a decrease in oxidant production and protects tissues against lipid peroxidation (Swaroo & Ramasarma 1985, Paller 1986). Therefore, it has been suggested that the hyperthyroid state predisposes tissues to oxidant-mediated injury, whereas hypothyroidism is thought to protect tissues against oxidant damage.

The inflammatory bowel diseases (IBD), namely ulcerative colitis and Crohn’s disease, are two chronic idiopathic diseases characterized by prominent intestinal inflammation. Among several experimental models of IBD, administration of an enema containing the contact sensitizing allergen trinitrobenzene sulfonic acid (TNBS) in ethanol produces an acute inflammation, which progresses to a chronic stage, that is morphologically similar to Crohn’s disease (Elson et al. 1995). Despite the extensive literature related to the role of oxidant/antioxidant status in the pathogenesis of inflammatory bowel disease, little is known about the endocrine control of mucosal integrity in these inflammatory conditions and associated gastrointestinal dysfunction. Since decreased thyroid hormone production is associated with a variety of immunological manifestations and antithyroid drugs were found to be useful in the treatment of several autoimmune diseases (Singer et al. 1994), the potential beneficial effects of the antithyroid drugs were tested to manipulate colitis (Oren et al. 1997). Although methimazole and propylthiouracil were shown to reduce mucosal damage in a rat model of colitis, their mode of action was not demonstrated.
The most common gastrointestinal symptoms in hypothyroid patients include diarrhea, malabsorption and steatorrhea while those in hypothyroid patients are constipation and gas retention (Sharma et al. 1995). Shafer et al. (1984) have suggested that abnormal gut motility is the primary cause of gastrointestinal manifestations in hypo/hyperthyroidism. On the other hand, colitis is associated with alterations in motor function, which varies with disease activity (Goldhill et al. 1999). As inflammation progresses, there are changes in the profile of inflammatory/immune cells and associated mediators that may directly or indirectly affect smooth muscle contractility. However, the effect of antithyroid drugs on colitis-induced gastrointestinal dysmotility has not previously been tested.

Thus, the aim of this study was to evaluate the putative beneficial effects of an antithyroidal drug, methimazole, on oxidative colonic injury and dysmotility in an experimental rat colitis model.

Materials and Methods

Animals

Wistar albino rats of both sexes (220–280 g) were kept in a room at a constant temperature of 22 ± 1 °C with a 12-h light/12-h darkness cycle and fed standard pellet chow and water which were available ad libitum. This study was approved by Marmara University School of Medicine, Animal Care and Use Committee.

Methimazole (0·04%) was administered in drinking water starting 15 days prior to induction of colitis, while control animals were treated with normal tap water for the same period of time (control group). Each group consisted of 7 animals.

Induction of colitis and determination of colonic damage and fecal output

Two weeks after the induction of hypothyroidism, inflammation was induced in the colon, under light ether anesthesia, by a single intracolonic administration of 0·8 ml of a 30 mg/ml trinitrobenzene sulfonic acid solution dissolved in 38% ethanol in saline introduced via an 8-cm cannula (Morris et al.1989). Comparisons were carried out with rats administered an equal volume of saline solution intracolonically (control group). On the 6th day following the induction of colitis, stool volume (mg/day) and number of fecal pellets (number/day) were measured for a 24-h period. For each animal, average stool weight (mg/number) was calculated and expressed as ‘fecal output’. Seven days after the induction of colitis, the rats were decapitated.

Trunk blood was collected and the serum samples were stored for the determination of tri-iodothyronine (T₃) and thyroxine (T₄) levels using radioimmunoassay. The last 8 cm of the colon was excised, opened longitudinally, and rinsed with saline solution. The distal colon was weighed and the mucosal lesions were examined macroscopically by a blinded observer using the criteria outlined in Table 1 (Wallace et al. 1989). Then, the colonic samples were stored at –80 °C for the measurement of lipid peroxidation and glutathione levels.

Determination of lipid peroxidation and glutathione levels

Tissue samples were homogenized in 10 ml vol. of ice-cold 10% trichloracetic acid, in an Ultra Turrax tissue homogenizer. Homogenized tissue samples were centrifuged at 3000 r.p.m. for 15 min at 4 °C. The supernatant was removed and recentrifuged at 15 000 r.p.m. for 8 min. Glutathione measurements were performed using a modification of the Ellman procedure (Aykaç et al. 1985). Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid-reactive substances as described previously. Lipid peroxide levels were expressed in nmol malondialdehyde (MDA) per g tissue (Casini et al. 1986).

Statistical analysis

Data were expressed as means ± S.E.M. Groups of data were analyzed by one-way analysis of variance followed by Tukey–Kramer multiple-comparisons test. Calculations were performed using an Instat statistical analysis package (GraphPad Software, San Diego, CA, USA). P values <0.05 were regarded as significant.

Results

Methimazole-treated rats were hypothyroid, as confirmed by serum T₃ and T₄ values (25·5 ± 0·29 ng/dl and 0·49 ± 0·01 ng/dl respectively) which were significantly...
lower than those of control rats (54·75 ± 0·75 ng/dl and 6·48 ± 0·46 ng/dl respectively; P<0·001).

Macroscopic damage score
The macroscopic damage score in the euthyroid colitis group (7·57 ± 0·2) was found to be significantly higher than that of the control group (0·1 ± 0·1; P<0·001), while the colitis score was reduced when the rats were hypothyroid (3·66 ± 0·6; P<0·001) (Fig. 1).

Colonic wet weight
The colonic wet weight (g/100 g body weight) of the euthyroid-colitis group (0·99 ± 0·07 g) was significantly higher than that of the control group (0·21 ± 0·01 g; P<0·001). Methimazole pretreatment before the induction of colitis markedly diminished the wet weight of colonic tissue (0·61 ± 0·06 g; P<0·01).

Lipid peroxidation and glutathione levels
Colonic lipid peroxidation level in the colitis group (42·9 ± 1·01 nmol MDA/g) was significantly higher than that in the control group (32·2 ± 1·10 nmol MDA/g; P<0·05). In hypothyroid rats with colitis, the lipid peroxidation level (30·43 ± 4·10 nmol MDA/g) was not significantly different from the control group (Fig. 2). The colonic glutathione level was decreased in the colitis group (0·64 ± 0·09 µmol/g) as compared with controls (2·94 ± 0·16 µmol/g; P<0·001), while this reduction was completely abolished in hypothyroid rats with colitis (2·49 ± 0·14 µmol/g; P<0·001) (Fig. 3).

Fecal output
Induction of colitis increased the daily stool volume (12·0 ± 0·6 g/day; P<0·01), without any effect on the daily fecal pellet number (40·1 ± 1·6/day), when
compared with the control group (6.6 ± 0.5 g/day and 38.3 ± 3.1 g/day respectively). Since the volume of fecal pellets was reduced in colitis without a change in number, a significant increase in average fecal output (300.0 ± 7.2 mg/pellet) was observed as compared with the control group (179.0 ± 10.1 mg/pellet; \( P < 0.05 \)) (Fig. 4). While administration of methimazole for two weeks had no significant effect on daily fecal output (281.0 ± 22.2 mg/pellet, not shown on the Figure) with respect to the control group, a reduction in the number of fecal pellets was observed (26.8 ± 1.5/day; \( P < 0.01 \)). However, methimazole in the colitis group exaggerated the fecal output (657.7 ± 103.7 mg/pellet; \( P < 0.001 \)) as shown by the increased daily stool volume (13.2 ± 1.7 g/day), despite the reduction in the number of fecal pellets (21.0 ± 1.7/day).

**Discussion**

The present study demonstrates that hypothyroidism induced by methimazole significantly ameliorates the inflammatory responses in TNBS-induced experimental colitis in the rat. This was shown by significant decreases in the macroscopic damage score and tissue wet weight in the hypothyroid group as compared with euthyroids. These data are in accordance with previous findings showing that the colonic damage was decreased by antithyroid drugs as shown by lesion score, tissue weight and myeloperoxidase staining both in early and late phases (Oren et al. 1997). Additionally, we observed that increased lipid peroxidation and glutathione utilization in the colitis group were reversed by methimazole treatment. These latter findings suggest that the colonic oxidant/antioxidant status has been altered by hypothyroidism.

Methimazole has frequently been used in the treatment of Graves’ disease and has been efficacious in experimental systemic lupus erythematosus in mice, both of which are autoimmune disorders (Singer et al. 1994). Several lines of evidence suggest that this mechanism of action may be attributed to the effect of hypothyroidism on cell-mediated immunity. For example, decreased thyroid function has been shown to be associated with CD4 T cell activation, increased number and activation of CD8 cells and decreased interleukin-2 (Volper 1994). Methimazole-induced hypothyroidism has been shown to suppress the expression of tumor necrosis factor (TNF) gene in peritoneal macrophages and to reduce alveolar macrophage production upon stimulation with lipopolysaccharide (Liu & Ng 1991, Liu 1993, Liu et al. 1993). However, in thioacetamide–induced hepatic failure, the suppression of TNF-α and other proinflammatory cytokines has not played a key role in the prevention of liver damage by hypothyroidism (Bruck et al. 1998). Thus, it was suggested that hypometabolism and decreased hepatocyte oxygen demand, associated with hypothyroidism, might be protective for the insulted liver (Bruck et al. 1998). Since methimazole ameliorates the TNBS-induced colonic damage, the results of the present study, taken together with the aforementioned studies, suggest that hypothyroidism might slow the colonic inflammatory processes by reducing the tissue oxygen demand, and some decrease in thyroid function appears to be beneficial for the inflamed colonic tissue.

One of the major effects of thyroid hormone is to increase mitochondrial respiration (Nishiki et al. 1978) which results in increased generation of reactive oxygen species, leading to oxidative damage to membrane lipids. There is a good deal of evidence to indicate that metabolic depression brought about by hypothyroidism is associated with a decrease in free radical production and a subsequent protection against lipid peroxidation (Asayama et al. 1987). This supports the notion that reduced demand for oxygen in hypothyroidism serves as a protective factor in tissue injury due to reactive oxygen metabolites. In agreement with these data, our results demonstrate that colitis induces oxidative damage associated with a reduction in glutathione reserves, whereas hypothyroidism offers a protection against free radical damage with a greater response of the antioxidant systems. This in vivo finding raised the possibility that methimazole could have a direct antioxidant effect. In an attempt to clarify this possibility, we compared the luminol–enhanced chemiluminescence values of xanthine–xanthine oxidase in the absence and presence of methimazole (10^{-7}–10^{-3} M) and our results revealed that methimazole had no superoxide scavenging activity per se (authors’ unpublished observation). Therefore, the protective mechanism may be related to the effects of...
hypometabolism on the production of reactive oxygen intermediates.

Hypothyroid patients frequently complain of moderate or severe constipation and this hypomotility may well be an early physical manifestation of hypothyroidism (Rahman et al. 1991). On the other hand, prominent clinical features of IBD are diarrhea and abdominal cramping (Rao & Read 1990). It has been reported that thyrotoxicosis may exacerbate the diarrhea of colitis and/or may cause patients to be refractory to treatment (Jarnerot et al. 1975). Thus, it is likely that the antithyroid drugs have an inhibitory effect on colitis-induced hypermotility. In the present study, colitis led to increased daily fecal volume without a change in the frequency of defecation, which resulted in an increased average stool weight (volume/pellet). On the other hand, when methimazole was given to the colitis group, frequency of fecal discharge was reduced, while colitis-induced increase in daily stool volume was not altered; thus, the average stool weight in 24 h was found to be increased twofold. This finding suggests that antithyroid treatment may be beneficial in the alleviation of colitis-induced diarrhea, without an effect on total stool volume. Since methimazole was effective in improving the oxidant colonic injury, it appears that the changes in the profile of inflammatory/immune cells and associated mediators have affected the colonic contractile activity. Moreover, hypothyroidism may influence the function of the muscle cell indirectly, by affecting the composition of connective tissue and extracellular space, and/or directly, by altering metabolic pumps and movement of electrolytes (Kowalewski & Kolodej 1977).

Therefore, the present study demonstrates that methimazole reduces colonic oxidative injury. This protective mechanism may be due to hypometabolism, which is associated with a decrease in the production of reactive oxygen intermediates and an increase in the response of antioxidant defence systems. In conclusion, the concomitant presence of hypothyroidism or the use of antithyroid drugs in IBD might be protective against inflammatory challenges of the colon, while the diarrhea of colitis might be exacerbated.

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