Original article

Effects of trimetazidine in ethanol- and acetic acid-induced colitis: oxidant/anti-oxidant status

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Abstract

There is overwhelming evidence in favour of a significant role of reactive oxygen metabolites (ROM) in the pathophysiology of inflammatory bowel disease (IBD) in man and in experimental animal models. This study was undertaken to investigate the possible protective effects of pretreatment with trimetazidine (TMZ) on the oxidant–anti-oxidant balance in ethanol- and acetic acid-induced colonic damage in rats. TMZ was chosen because of its various cytoprotective features (preserving cellular ATP levels, limiting intracellular acidosis and limiting inorganic phosphate, Na^+ and Ca^{2+} accumulation) and anti-oxy characteristics which were previously reported. A total of 80 rats were randomized into eight major groups each consisting of 10 animals. Animals in groups 1, 2 and 3 served as models of ethanol-induced colitis (0.25 ml of 30% (v/v) ethanol), while group 4 served as their control. Animals in groups 5, 6 and 7 served as models of acetic acid-induced colitis (1 ml of 4% (v/v) acetic acid), while group 8 served as their control. TMZ was administered 5 mg/kg by intrarectal (i.r.) and intraperitoneal (i.p.) routes to groups 1, 2, 5 and 6. Intraperitoneal administration of TMZ was used in order to evaluate its systemic effect while i.r. administration was used to determine its local effect. After decapitation, colon mucosa samples were obtained and evaluated macroscopically and microscopically. Myeloperoxidase (MPO) activities as markers for inflammation, malondialdehyde (MDA) levels as markers for oxidant stress and reduced glutathione (GSH) and oxidized glutathione (GSSG) levels as markers for anti-oxidant status were determined. Acute colitis was observed in macroscopic and microscopic evaluation in ethanol- and acetic acid-administered groups compared with controls ($P = 0.000$). The macroscopic and microscopic scores in colitis groups were correlated with MPO activities ($r = 0.5365$, $P = 0.000$ and $r = 0.5499$, $P = 0.000$, respectively). MDA and GSSG levels in the acetic acid-induced colitis group were higher compared with ethanol-induced colitis group ($P < 0.008$ and $P < 0.005$, respectively), while GSH levels were significantly lower ($P < 0.05$). While TMZ pretreatment did not improve the oxidant state, it preserved the GSH levels significantly ($P < 0.05$). In conclusion, ethanol- and acetic acid-induced colitis models are appropriate experimental colitis models which in many ways manifest the characteristics seen in tissue injury related to colitis in humans. Of these two, the acetic acid-induced colitis model proved more suitable than the ethanol model for investigating the alterations in long-term and in more severe tissue injury. While TMZ pretreatment via i.p. or i.r. route did not improve the oxidative-inflammative state in either of these models, it did contribute significantly to the preservation of the anti-oxidant pool via the conservation of intracellular GSH levels. This conserving effect of TMZ was substantially more pronounced in the i.p. route compared with the i.r. route. Based on our results, we conclude that the 'GSH-preservation' role of TMZ can be the mode of action it manifests as an anti-oxy compound.

Keywords Acute colitis models, myeloperoxidase, oxidant stress, glutathione status, trimetazidine, rats

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Introduction

There is overwhelming evidence in favour of a significant role of reactive oxygen metabolites (ROM) in the pathophysiology of inflammatory bowel disease (IBD) in man [1] and in experimental animal models [1–3]. Although the initial mechanism is unknown, it has been hypothesized that the infiltrating macrophages and neutrophils expose the inflamed intestine to substantial oxidative stress by excessive production of ROM, thus causing an imbalance between oxidant and anti-oxidant systems in the colon [1,2,4].

Experimental models in which colitis is induced by chemicals or haptens have been widely used to understand the mechanisms involved in the disease state with the goal of discovering new therapeutic agents [2,4,5]. Among these experimental models, intrarectal administrations of organic acids (acetic acid) or solvents (ethanol) are known to create initial injurious effects to the colonic mucosa, a process which produces acute colitis. This initial caustic injury is well presented by the dramatic increase in mucosal permeability and tissue water content and by histological appearance. These developments are then followed by signs of classical inflammation such as neutrophil accumulation, widespread oedema and haemorrhage, hyperaemia, ulceration and necrosis.

New therapeutic strategies to treat IBD are needed because (i) therapies currently in use have produced unsatisfactory or only partially effective results, and (ii) in many cases high doses of corticosteroids are required to treat patients. This study was undertaken to investigate the possible protective effects of pretreatment with such a new agent, trimetazidine (TMZ; 1-[2,3,4-trimethoxybenzyl] piperazine HCl), on the oxidant–anti-oxidant balance in ethanol- and acetic acid-induced colonic damage in rats. TMZ was chosen because of its various cytoprotective features (preserving cellular ATP levels, limiting intracellular acidosis and limiting inorganic phosphate, Na^+ and Ca^{2+} accumulation) on ischaemic cardiac injury, although it was originally introduced only as an anti-anginal compound [6,7]. Through recent research on TMZ it was noted that these effects were independent of alterations in oxygen supply or demand, since neither systemic haemodynamics nor coronary blood flow were altered by the compound [8]. TMZ was also shown to limit membrane damage induced by ROM, inhibit neutrophil infiltration into reperfused myocardium, as well as function as an anti-oxy radical compound in conditions of increased oxy radical production. These characteristics of TMZ create a free radical scavenging activity which might explain its cardioprotective role [9,10]. Although TMZ's cytoprotective role has been investigated in various cardiac, nephrotic, respiratory or neurologic diseases, our study is the first to assess its effects on experimental colitis.

Materials and methods

Animals

Male Sprague-Dawley rats weighing 180–220 g were obtained from Ege University Experimental Research Center (Izmir, Turkey). They were held in stainless steel cages. The animal room was maintained on a 12 h light/12 h dark cycle and at 21–22 °C. The rats were fasted overnight with access to water *ad libitum*.

The study was approved by the Animal Research Ethics Committee, Ege University, School of Medicine.

Drugs

Ethanol (98%) was obtained from Izmir Tekel Organization (Izmir, Turkey), while acetic acid was provided by Sigma Chemical Co. Inc. (Tokra Medical, Izmir, Turkey). TMZ was supplied by Servier Drug Co. (Istanbul, Turkey).

Colitis induction and treatment schedules

A total of 80 rats were randomized into eight major groups each consisting of 10 animals. Animals in groups 1, 2 and 3 served as models of ethanol-induced colitis, while group 4 served as their control. Animals in groups 5, 6 and 7 served as models of acetic acid-induced colitis, while group 8 served as their control. The animals in groups 1 and 2 received TMZ in doses of 5 mg/kg by the intrarectal (i.r.) and intraperitoneal (i.p.) routes, respectively. Groups 3 (placebo) and 4 received only 1 ml of saline (i.p.) instead of TMZ. Similarly, animals in groups 5 and 6 received TMZ in doses of 5 mg/kg by the i.p. and i.r. routes, respectively, while groups 7 (placebo) and 8 received only 1 ml of saline (i.p.) instead of TMZ. One hour after the administration of TMZ, the animals were lightly anaesthetized with ether and diffuse acute colitis was induced by either i.r. administration of 0.25 ml of 30% (v/v) ethanol (groups 1, 2 and 3) [5] or i.r. administration of 1 ml of 4% (v/v) acetic acid pH 2.3 (groups 5, 6 and 7) [4] using a 1-ml syringe fitted with a 6F paediatric catheter. The solutions were administered slowly (in 15 s) into the lumen of the colon and after 20 s of exposure they were withdrawn. The lumen was then flushed with 1.5 ml of PBS pH 7.4. The control groups (groups 4 and 8) received i.r. saline (0.25 ml and 1 ml, respectively) and saline was withdrawn in the same way.

The rats in the ethanol-induced colitis group and their controls (groups 1, 2, 3 and 4) were killed by cervical dislocation 10 min after the administration of ethanol

[5], while rats in the acetic acid-induced colitis group and their controls (groups 5, 6, 7 and 8) were killed in the same way 48 h later [4].

Morphological studies

The segment of colon extending from the anus to the splenic flexure was excised and opened by a longitudinal incision. Mucosal injury was assessed using the grading scale of Wallace *et al.* [5] as presented in Table 1.

For histological analysis, colonic samples obtained from each rat were immersed in 10% buffered formalin pH 7. The samples were processed by routine techniques and wax-embedded for light microscopy and stained with haematoxylin and eosin. Sections of each sample were examined at a magnification of ×200. Scoring criteria were based on the method of Noronha-Blob *et al.* [11], in which inflammation was graded on a 0–4 scale as presented in Table 2.

Biochemical studies

Myeloperoxidase (MPO) activities were determined by the method of Krawisz *et al.* [12] and results were expressed as U/g wet tissue. Colonic tissue malondialdehyde (MDA) levels were determined spectrophotometrically as thiobarbituric acid reactive substances using the method of Ohkawa *et al.* [13], slightly modified by Rungby *et al.* [14]. Results were expressed as nmol/g wet tissue. Glutathione (GSH) activities were determined by the enzymatic recycling method of Teare *et al.* [15], in which the reduced form of glutathione was masked with 2-vinylpyridine in order to measure the oxidized glutathione.

Statistical analysis

Non-parametric statistics were used to evaluate the results. *Post hoc* analysis of one-way analysis of variance (ANOVA) for independent samples was used for comparison as appropriate.

Table 1 Macroscopic mucosal injury grading (according to Wallace *et al.*) [5].

Normal mucosa	Grade 0
Diffuse patches of superficial hyperaemia	Grade 1
Patches of severe hyperaemia and normal mucosa	Grade 2
Extensive hyperaemia and haemorrhage	Grade 3

Results

In our study, degrees of colonic damage induced by ethanol or acetic acid were assessed macroscopically and histologically as well as biochemically, using MPO as an inflammation marker, MDA as a lipid peroxidation parameter and oxidative stress marker, total glutathione (tGSH), reduced glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG as anti-oxidant status markers.

Macroscopic and microscopic damage

We observed that colon mucosa samples of rats administered i.r. ethanol and acetic acid were characterized by extensive vasocongestion and haemorrhage which was not observed in rats given saline alone (Fig. 1). The macroscopic scores of the groups showed a significant difference ($P < 0.0001$). Average macroscopic scores of 1.00 ± 0.67 and 2.50 ± 0.71 were determined for the ethanol- and acetic acid-induced colitis placebo groups, respectively, compared with scores of 0.00 and 0.00 in groups given saline alone. Pretreatment with TMZ (both i.r. and i.p. routes) did not improve the macroscopic

Figure 1 Macroscopic appearance of different grades of colon samples from experimental groups (starting from the top, macroscopic scores of 0, 3, 1 and 5 are seen).

Table 2 Microscopic mucosal injury scoring (according to Noronha-Blob).

Intact epithelium, no leucocytes or haemorrhage	Score 0
<25% disrupted epithelium, focal leucocyte infiltrates and focal haemorrhage	Score 1
25% disrupted epithelium, focal leucocyte infiltrates and focal haemorrhage	Score 2
≤50% disrupted epithelium, widespread leucocytes and haemorrhage	Score 3
>50% disrupted epithelium, extensive leucocyte infiltration and haemorrhage	Score 4

scores as there was no significant difference between the treated and non-treated groups.

Microscopically, the intense inflammation of the colon was characterized by infiltration of neutrophils primarily into the mucosa and submucosa, widespread oedema, haemorrhage and necrosis (Fig. 2). From histological evaluation of injury, average microscopic scores of 2.3 ± 0.15 and 2.6 ± 0.16 were determined for the ethanol- and acetic acid-induced colitis placebo groups, respectively, compared with a score of 0.10 ± 0.32 and 0.20 ± 0.42 in groups given saline alone. The severity of colonic damage was more pronounced in the acetic acid-induced colitis groups and pretreatment with TMZ (both i.r. and i.p. routes) was not effective in any of the colitis groups. Related data about macroscopic and microscopic scores are presented in Table 3.

MPO

MPO activity as marker for neutrophil accumulation showed significant changes within the groups (Fig. 3). Ethanol- and acetic acid-induced colitis placebo groups showed significantly higher values (28.50 ± 5.99 and 55.50 ± 12.15 U/g tissue, respectively) compared with their controls (16.60 ± 4.58 and 19.40 ± 5.74 U/g tissue). However, pretreatment with TMZ did not cause any significant alterations among the treated and non-treated colitis groups with regard to MPO activity. It was notable that MPO activity in the acetic acid-induced colitis group was higher compared with the ethanol-induced colitis group and that MPO activity was significantly correlated to macroscopic ($r = 0.5365$, $P < 0.001$) and microscopic ($r = 0.5499$, $P < 0.001$) scores of damage.

Lipid peroxidation

MDA was assessed as the indicator for lipid peroxidation involved in our experimentally produced colitis models. Although the levels of MDA were higher in all colitis-induced groups than in their controls, this difference was not statistically significant (Fig. 4). It was notable that MDA levels in the acetic acid-induced colitis groups were higher compared with the ethanol induced-colitis groups, and that microscopic scores of damage were correlated to MDA levels ($r = 0.2989$, $P = 0.008$). Pretreatment with TMZ (both i.r. and i.p. routes) did not improve the MDA levels in either colitis group. Similarly, there was not a significant difference in the effects of using either the i.p. or i.r. routes.

Anti-oxidant status

The tGSH, GSH, GSSG and GSH/GSSG results are presented in Table 4. There was a significant difference in tGSH ($P = 0.002$), GSH ($P = 0.0008$), GSSG ($P < 0.001$), GSH/GSSG ($P = 0.0002$) values between the groups by one-way ANOVA test.

As the results in Table 4 indicate, GSH levels in the TMZ pretreated (i.r.) ethanol-induced colitis group were significantly higher ($P < 0.05$) than GSH levels in the TMZ pretreated (i.r.) acetic acid-induced colitis group. This observation reaffirms the more pronounced colitis in the acetic acid group. The significant difference ($P < 0.05$) in GSH levels between the i.r. and i.p. administered TMZ pretreatment acetic acid-induced colitis groups indicates that TMZ has a more protective effect via the i.p. route than via the i.r. route.

Table 4 also reveals significantly higher ($P < 0.05$) levels of GSSG in the i.r. route TMZ pretreated ethanol-induced colitis group compared with the i.p. route group. These results again suggest that i.p. administration of TMZ has a more protective role in colitis. Similarly, the significantly higher levels of GSSG found in TMZ pretreatment (i.p.) acetic acid-induced colitis group compared with TMZ pretreatment (i.p.) ethanol-induced colitis group show that acetic acid causes a more pronounced injury compared with ethanol.

The GSH/GSSG ratio was also significantly lower in TMZ pretreatment (i.p.) acetic acid-induced colitis group than in the TMZ pretreatment (i.p.) ethanol-induced colitis group, because of the high levels of GSSG found in the previous group. There was also a significant difference in GSH/GSSG ratio between the TMZ pretreated (i.p.) ethanol-induced colitis group compared with its control group. These significant differences indicate that TMZ has a more protective role when administered via the i.p. route.

Discussion

Both acetic acid and ethanol administration are widely used as models of IBD because of their simplicity, adaptability to small animals, reproducibility and non-invasive nature, as well as having similarities to the human conditions with respect to arachidonate metabolism and histopathological features [3,4,5,16].

As a colitis-producing agent, the 'barrier breaker ethanol' is an extremely potent proinflammatory solvent [5]. The nature of its damage to the colonic mucosa is characterized by vascular congestion and extensive epithelial disruption, but mucosal injury produced by the barrier breaker ethanol resolves quickly. In contrast, acetic acid is considered responsible for various damaging mechanisms of action such as the initiation of a non-specific acid-induced injury to the colonic mucosa followed by an acute inflammatory response [3], or damage through other pathophysiological events

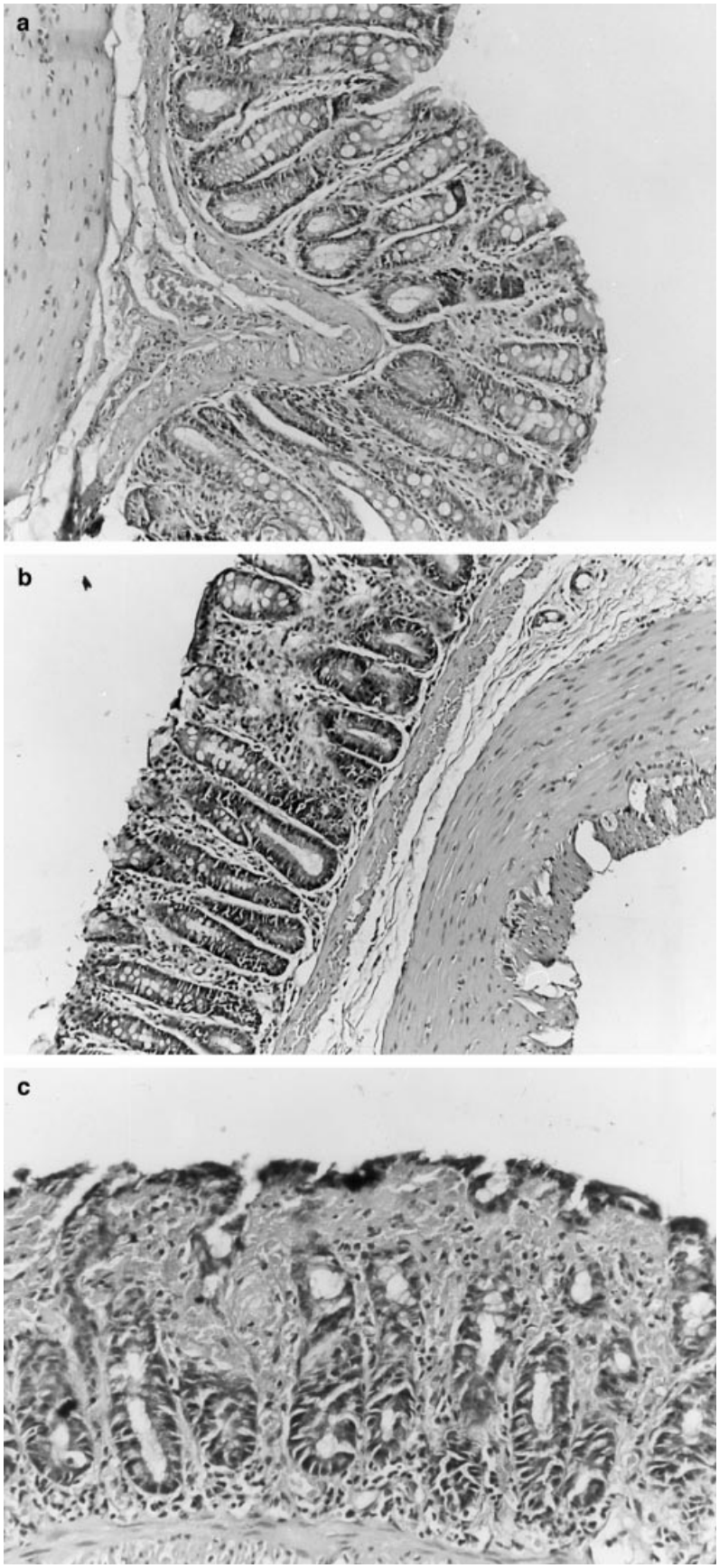

Figure 2 Microscopic appearance of colon samples from experimental groups. (a) Score 2, diffuse inflammatory cells. (b,c) Score 3, marked inflammatory focal leucocyte infiltration, presence of haemorrhage.

Table 3 Histopathologic evaluation of the effects of ethanol and acetic acid on colon mucosa.

Study groups	Macroscopic score (mean ± s.d.) Wallace [5]	Microscopic score (mean ± s.d.) Noronha-Blob [11]
Group 1 (n = 10) (i.r. TMZ + i.r. ethanol)	1.10 ± 0.74*	2.20 ± 0.42
Group 2 (n = 10) (i.p. TMZ + i.r. ethanol)	1.00 ± 0.67**	2.10 ± 0.74
Group 3 (n = 10) (i.p. saline + i.r. ethanol)	1.00 ± 0.67**	2.30 ± 0.48
Group 4 (n = 10) (i.r. saline + i.r. saline)	0.00	0.10 ± 0.32
Group 5 (n = 10) (i.p. TMZ + i.r. acetic acid)	2.11 ± 0.78***	3.22 ± 0.67
Group 6 (n = 10) (i.r. TMZ + i.r. acetic acid)	1.70 ± 1.06**	2.40 ± 0.52
Group 7 (n = 10) (i.p. saline + i.r. acetic acid)	2.50 ± 0.71****	2.60 ± 0.52
Group 8 (n = 10) (i.r. saline + i.r. saline)	0.00	0.20 ± 0.42

*Significantly different from groups 4 and 6; **significantly different only from groups 4 and 8 (controls); ***significantly different from groups 1, 2, 3, 4 and 8; ****significantly different from groups 1, 2, 3, 4, 6 and 8.

(e.g. fluid and electrolyte secretion) which have been observed when non-cytotoxic concentrations of the acid were used [16]. Whatever the mechanism of action is, the protonated form of the acid liberates protons within the intracellular space, causing a massive intracellular acidification resulting in massive epithelial damage.

Our results also demonstrate that i.r. administration of ethanol or acetic acid results in diffused colitis followed by inflammation. The damage induced by the agents is characterized by marked thickening of the colonic wall, vascular congestion, oedema and infiltration of neutrophils which was assessed histologically and enzymatically.

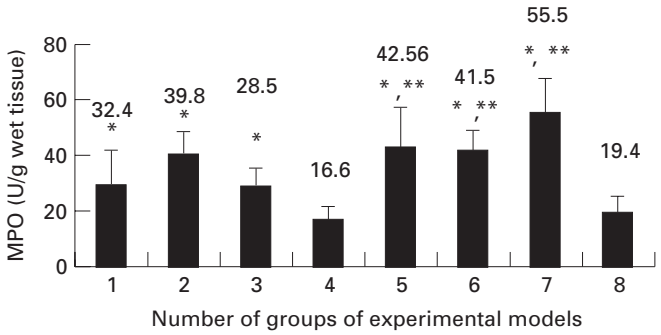

Figure 3 Myeloperoxidase (MPO) levels (U/g wet tissue) (mean ± s.d.) of colon mucosa in experimentally induced colitis models. Group 1 (n = 10) (i.r. TMZ + i.r. ethanol); group 2 (n = 10) (i.p. TMZ + i.r. ethanol); group 3 (n = 10) (i.p. saline + i.r. ethanol); group 4 (n = 10) (i.p. saline + i.r. saline); group 5 (n = 10) (i.p. TMZ + i.r. acetic acid); group 6 (n = 10) (i.r. TMZ + i.r. acetic acid); group 7 (n = 10) (i.p. saline + i.r. acetic acid); group 8 (n = 10) (i.p. saline + i.r. saline). *Significantly different from the controls. **Marked protective effect of TMZ when compared with the damage of acetic acid alone. Significance level is considered as $P < 0.050$ according to multiple range tests: Student–Newman –Kuels.

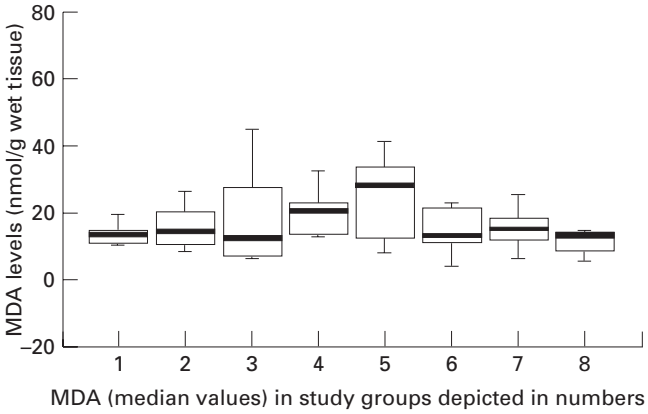

Figure 4 Malondialdehyde (MDA) levels (nmol/g wet tissue) (median) of colon mucosa in experimentally induced colitis models. Group 1 ($n=10$) (i.r. TMZ + i.r. ethanol); group 2 ($n=10$) (i.p. TMZ + i.r. ethanol); group 3 ($n=10$) (i.p. saline + i.r. ethanol); group 4 ($n=10$) (i.p. saline + i.r. saline); group 5 ($n=10$) (i.p. TMZ + i.r. acetic acid); group 6 ($n=10$) (i.r. TMZ + i.r. acetic acid); group 7 ($n=10$) (i.p. saline + i.r. acetic acid); group 8 ($n=10$) (i.p. saline + i.r. saline).

Table 4 Comparison of glutathione status in (**a**) ethanol-induced colitis group, (**b**) acetic acid-induced colitis group.
a. Ethanol group

Study groups	tGSH (nmol/gww)	GSH (nmol/gww)	GS2SG (nmol/gww)	GSH/GSSG
Group 1 (n = 10) i.r. TMZ + i.r. ethanol	393.56 ± 89.39	371.08 ± 92.57	22.48 ± 14.85	27.00 ± 25.21
Group 2 (n = 10) i.p. TMZ + i.r. ethanol	316.28 ± 115.20	306.98 ± 114.51	9.30 ± 4.72	42.57 ± 28.05
Group 3 (n = 10) i.p. saline + i.r. ethanol	352.28 ± 119.16	337.33 ± 125.27	14.95 ± 12.76	43.39 ± 38.17
Group 4 (n = 10) IP saline + IR saline	398.40 ± 99.00	365.67 ± 97.47	32.73 ± 9.02	12.32 ± 5.91

b. Acetic acid group

Study groups	tGSH (nmol/gww)	GSH (nmol/gww)	GSSG (nmol/gww)	GSH/GSSG
Group 6 (n = 10) i.r. TMZ + i.r. acetic acid	191.51 ± 90.34*	170.71*** ± 89.87	20.80 ± 7.22	9.59 ± 7.77
Group 5 (n = 10) i.p. TMZ + i.r. acetic acid	341.23** ± 106.73	306.38 ± 102.24	34.84**** ± 8.31	8.95***** ± 2.51
Group 7 (n = 10) i.p. saline + i.r. acetic acid	251.43 ± 75.46	233.41 ± 74.84	18.02 ± 5.99	14.60 ± 7.52
Group 8 (n = 10) i.p. saline + i.r. saline	306.23 ± 63.39	265.08 ± 69.29	21.15 ± 5.03	13.08 ± 4.24

Values represent mean ± s.d.
Significance level is considered as $P < 0.05$ according to multiple range tests: Student–Newman–Kuels.
*Total glutathione (tGSH) values of group 6 are significantly different from the values of group 1 and both of the saline (control) groups; **tGSH values of group 5 are significantly different from group 6, indicating the presence of marked i.p. effect of TMZ; ***GSH values of group 6 are significantly different from group 1; ****oxidized glutathione (GSSG) values are significantly higher in group 5 than in group 2, revealing that TMZ administration is not effective in the acetic acid group as it is in the ethanol group; *****GSH/GSSG values are significantly lower in group 5 than in group 2, reaffirming that the effect of TMZ on GSH/GSSG status is more obvious in the ethanol group.

MPO is an enzyme found predominantly in neutrophils and has been used as an effective quantitative index of inflammation due to the strong correlation between MPO activities and the histological analysis of neutrophil infiltration of the colon [11]. The significantly higher macroscopic and microscopic scores as well as MPO

activity and MDA levels in the acetic acid-induced colitis group all indicate the more diffuse and progressive damage produced by the use of acetic acid. These results suggest that while ethanol-induced injury is prone to resolve quickly, acetic acid-induced injury is more extensive and longer lasting, creating severe inflammation secondary to the initial injury.

Tissue damage in colitis is due, at least in part, to oxidative stress from the ROM which are produced by the granulocytes that enter the mucosa during inflammation. Although the initial stimulus provoking inflammation of the colon mucosa is not firmly established in humans, acetic acid and ethanol in our experimental models produced intracellular acidification and epithelial damage, respectively, which probably accounts for the initiation of inflammation. Neutrophil accumulation in the inflammation site, as assessed histologically, may account for the hyperaemia associated with colonic inflammation via neutrophil-derived factors. It is known that activated neutrophils release a variety of substances (some of the leukotrienes and prostaglandins) that influence basal vascular tone. ROM that are produced during the respiratory burst have also been shown to produce vasodilation of both arterioles and venules. As a result, in sites of mucosal inflammation it is probable that the interaction of leucocyte-generated superoxide radical formation in proximity to relatively high concentrations of low molecular weight chelate iron plays a key role in stimulating the amplification of the inflammatory response and the acceleration of mucosal hyperaemia and haemorrhage observed characteristically in colitis. The flared inflammatory response with excessive production of ROM is then responsible for causing cytotoxicity through sulfhydryl oxidation, cytochrome inactivation and degradation of proteins and lipids [1].

The results of our study indicate that in both the acetic acid- and ethanol-induced models of colitis there is increased lipid peroxidation as revealed by increased levels of MDA. It was notable that these changes were more prominent with acetic acid administration, which may be explained by the information presented above. Since the rats in the acetic acid group were decapitated 48 h after the administration of the agent, it was possible to observe the more severe damage as the sharper increases in MDA levels and MPO activity.

Glutathione, the most important intracellular thiol in living systems, was also assessed in our study in four indices: GSH, GSSG, GSH/GSSG ratio and tGSH. It is well known that, at physiological ranges, the GSH status is maintained in its reduced state which is controlled by the GSH-peroxidase and reductase systems connected with the $NADP^+$/NADPH redox pair. Endogenous H_2O_2 oxidizes GSH to GSSG, catalysed by GSH-peroxidase. Depending on the availability of NADPH, GSSG is reduced back to GSH by GSSG reductase. During an oxidative stress, there will be flux of glutathione to the oxidized form. The ratio of reduced to oxidized glutathione may then be an indication that this oxidative stress has occurred. Similarly, Holmes *et al.* have shown that changes in mucosal GSSG and the GSH/GSSG ratio are correlated with clinical and histological measures of disease activity in active colitis [17]. While these changes appeared to be a consequence rather than the cause of inflammation, the results were consistent with two important indicators: (i) the presence of oxidative damage in active colitis as well as the relationship between the redox status of the mucosal glutathione system, and (ii) the severity of acute inflammation in this disorder.

The above mentioned changes in anti-oxidant status were also assessed in our experimental work as evidenced by the reduction in GSH levels, the GSH/GGSG ratios, tGSH levels and the increases in GSSG in both models of colitis compared with their controls. The accumulated GSSG in the cell is a sensitive index of oxidative stress *in vivo*. The altered $NADP^+$/NADPH ratio in the inflamed site in colitis models probably serves as the reason for the increase in GSSG, since GSSG reductase is dependent on the availability of NADPH.

To date, numerous studies have reported that adding TMZ to the perfusion fluid of ischaemic rat hearts significantly improves the energy state of the myocardial cells, and limits the neutrophil infiltration and the consequent membrane damage [6,7]. Our group's previous report has also shown that although administration of TMZ does not provoke any significant alterations in limiting oxyradical production, it preserves the tGSH levels of the cell, probably by improving the energy status [18]. Similarly, pretreatment with TMZ in this experimental colitis model produced no improvements in macroscopic and microscopic scores, MDA levels or MPO activity, all of which indicate the relative degree of tissue injury. The only significant difference was detected in GSH levels. TMZ pretreatment in the ethanol-induced colitis group caused higher GSH levels, lower GSSG levels and higher GSH/GSSG ratios compared with the respective levels in acetic acid-induced colitis. These results indicate that pretreatment with TMZ can be effective in the initiation of the injury. The results also indicate that administering TMZ via the i.p. route results in a more pronounced glutathione-preserving effect than using the i.r. route. This conclusion is confirmed by the higher levels of GSH in the i.p. route groups. Although i.p. and i.r. routes are both conventional drug application routes, it is well known that the i.p. route has a more pronounced local effect while the i.r. route has an

additional systemic effect. Additionally, while drug absorption is via active diffusion in the i.p. route, it is mainly via passive diffusion in the i.r. route, hence open to many interfering factors (the lipid/water partition coefficient of the drug, molecular structure and size, state of crystalization, its ability to form complexes, etc.). Due to these aspects, it is reasonable to expect that the i.p. route would mediate a higher conservative response in intracellular GSH levels. TMZ, probably by improving the energy state of the cells and by limiting neutrophil accumulation, conserved the $NADP^+$/NADPH redox pair which then improved the activity of GSSG reductase shown by the conservation of GSH.

In conclusion, ethanol- and acetic acid-induced colitis models are appropriate experimental colitis models which in many ways manifest the characteristics seen in tissue injury related to colitis in humans. Of these two, the acetic acid-induced colitis model proved more suitable than the ethanol model for investigating the alterations in long-term and in more severe tissue injury. While TMZ pretreatment via i.p. or i.r. route in both of these models did not improve the oxidative-inflammative state, as shown by the lack of differences in macroscopic and microscopic scores, MDA levels and MPO activities in groups with or without treatment, it did contribute significantly to the preservation of the anti-oxidant pool via the conservation of intracellular GSH levels. This conserving effect of TMZ was substantially more pronounced in the i.p. route compared with the i.r. route. Based on our results, we conclude that the 'GSH-preservation' role of TMZ can be the mode of action it manifests as an anti-oxy compound.

References

1 Babbs CF. Oxygen radicals in ulcerative colitis. *Free Rad Biol Med* 1992; **13:** 169–81.

2 Ondrula D, Nelson RL, Andrianopoulos G *et al.* Quantitative determination of pentane in exhaled air correlates with colonic inflammation in the rat colitis model. *Dis Colon Rectum* 1993; **36:** 457–62.

3 Yamada T, Marshall S, Specian R *et al.* A comparative analysis of two models of colitis in rats. *Gastroenterol* 1992; **102:** 1524–34.

4 Sekizuka E, Grisham MB, Li M *et al.* Inflammation induced intestinal hyperemia in the rat: role of neutrophils. *Gastroenterol* 1988; **95:** 1528–34.

5 Wallace JL, Whittle BJR, Boughton-Smith NK. Prostaglandin protection of rat colonic mucosa from damage induced by ethanol. *Dig Dis Sci* 1985; **30:** 866–76.

6 Lavanchy N, Martin J, Rossi A. Anti-ischemic effects of trimetazidine: ^{31}P NMR spectroscopy in the isolated rat heart. *Arch Int Pharmacodyn Ther* 1987; **286:** 97–110.

7 Kay L, Finelli C, Aussedat J *et al.* Improvement of long-term preservation of the isolated arrested rat hearts by trimetazidine: effects on the energy state and mitochondrial function. *Am J Cardiol* 1995; **76:** 45B–49B.

8 Kober G, Buck T, Sievert H *et al.* Myocardial protection during percutaneous transluminal coronary angioplasty: effects of trimetazidine. *Europ Heart J* 1992; **13:** 1109–15.

9 Knight C, Fox K. From antianginal drugs to myocardial cytoprotective agents. *Am J Cardiol* 1995; **76:** 4B–7B.

10 Maridonneau PI, Harpey C. Effect of trimetazidine on membrane damage induced by oxygen free radicals in human red cells. *Br J Clin Pharmacol* 1985; **20:** 148–51.

11 Noronha-Blob L, Lowe VC, Mulhauser RO *et al.* NPC15669, an inhibitor of neutrophil recruitment, is efficious in acetic acid-induced colitis in rats. *Gastroenterol* 1993; **104:** 1021–9.

12 Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. *Gastroenterol* 1984; **87:** 1344–50.

13 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95:** 351–8.

14 Rungby J, Flyvbjerg A, Andersen HB *et al.* Lipid peroxidation in experimental diabetes in rats: effects of diabetes and insulin. *Acta Endocrinol* 1992; **126:** 378–80.

15 Teare JP, Punchard NA, Powell JJ *et al.* Automated spectrophotometric method for determining oxidized and reduced glutathione in liver. *Clin Chem* 1993; **39:** 686–9.

16 Bustos-Fernandez K, Gonzales E, de Paolo IL *et al.* Organic anions induce colonic secretion. *Dig Dis Sci* 1976; **2:** 329–32.

17 Holmes EW, Yong SL, Eiznhamer D *et al.* Glutathione content of colonic mucosa. *Dig Dis Sci* 1998; **43:** 1088–95.

18 Girgin F, Tuzun S, Demir A, Kuralay F, Ozutemiz O, Tanyalcin T. Cytoprotective effects of trimetazidine in carmustine cholestasis. *Exp Toxicol Pathol* 1999; **51:** 326–9.

