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Oxidative damage and altered antioxidant enzyme activities in the small intestine of streptozotocin-induced diabetic rats

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Abstract

The small intestine exhibits numerous morphological and functional alterations during diabetes. Oxidative stress, a factor implicated in the pathogenesis of diabetic complications may contribute towards some of these alterations. We therefore investigated the occurrence of oxidative stress in the small intestine during diabetes by measuring the extent of oxidative damage as well as the status of the antioxidant defense system. Significant increases in lipid peroxidation (four-fold) as measured by TBARS and protein oxidation (38%) as measured by protein carbonyl content were observed after 6 weeks of diabetes. A distinct elevation in the activities of catalase (123.9%) and superoxide dismutase (71.9%) and a decline in the activity of glutathione peroxidase (67.7%) were also observed. The steady state mRNA levels of these enzymes measured by RT-PCR were, however, unchanged suggesting the absence of transcriptional control. In contrast, no changes in the levels of protein and non-protein thiols as well as the activities of glutathione reductase and glutathione-*S*-transferase were detected. Interestingly, decreases in the activities of xanthine oxidase (XO; 25.7%) and xanthine dehydrogenase (XDH; 42.6%) indicate that they do not contribute significantly to oxidative damage. The results thus reveal the occurrence of oxidative stress in the small intestine during diabetes and suggest its possible involvement in some of the accompanying functional alterations.

Keywords: Small intestine; Diabetes; Oxidative stress; Antioxidant enzymes; mRNA expression

1. Introduction

Diabetes mellitus results in severe metabolic imbalances and pathological changes in many tissues. Oxidative stress is believed to play a role in the development of complications in these tissues (Baynes & Thorpe, 1996). Recently, it has been proposed that carbonyl stress, i.e. the increase in reactive carbonyl compounds derived from oxidative and non-oxidative reactions, leads to increased chemical modification of proteins and at a later stage, to oxidative stress and tissue damage. A deficit in the detoxification of carbonyl compounds by the enzymes of the glyoxalase pathway and aldose reductase is believed to be partly responsible for carbonyl stress and consequent oxidative stress (Baynes & Thorpe, 1999).

The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress (Halliwell & Gutteridge, 1999). Alterations in the antioxidant enzyme activities and increased oxidative damage have been

Abbreviations: SOD, superoxide dismutase; GPx, glutathione peroxidase; GSH, glutathione; GR, glutathione reductase; GST, glutathione-*S*-transferase; ROS, reactive oxygen species; MDA, malondialdehyde; XO, xanthine oxidase; XDH, xanthine dehydrogenase; HNE, 4-hydroxynonenal; CuZnSOD, copper and zinc containing superoxide dismutase

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demonstrated in different tissues of diabetic animals (Kakkar, Kalra, Mantha, & Prasad, 1995). In addition to alterations in activities, changes in the mRNA expression of the antioxidant enzymes have also been reported (Cederberg, Galli, Luthman, & Eriksson, 2000; Kamata & Kobayashi, 1996; Reddi & Bollineni, 1997).

Diabetes is accompanied by several morphological and functional changes in the small intestinal mucosa (Brasitus & Dudeja, 1985; Fedorak, 1990; Feingold et al., 1982; Feingold, Moser, Adi, Soued, & Grunfeld, 1990; Sharma & Sivakami, 1998; Zoubi, Mayhew, & Sparrow, 1995). These include hyperplasia and hypertrophy of the epithelial cells (Zoubi et al., 1995), elevated levels of digestive enzymes (Sharma & Sivakami, 1998), increased absorption of sugars, amino acids (Fedorak, 1990), enhanced endogenous synthesis of cholesterol (Feingold et al., 1982) and triglycerides (Feingold et al., 1990) and decreased fluidity of the brush border membrane (Brasitus & Dudeja, 1985). The intestinal mucosa is also vulnerable to oxidative stress on account of the constant exposure to reactive oxygen species (ROS) generated by the luminal contents such as oxidized food debris, transition metals like iron and copper, bacterial metabolites, bile acids and salivary oxidants (Halliwell, Zhao, & Whiteman, 2000). Though ROS-mediated injury to the small intestine has been demonstrated in several conditions such as ischemia/reperfusion (Halliwell & Gutteridge, 1999), inflammatory bowel disease (Halliwell & Gutteridge, 1999), surgical stress (Prabhu, Anup, & Balasubramanian, 2000), radiation enteritis (Mutlu-Turkoglu et al., 2000), iron supplementation (Srigiridhar & Nair, 1998) and zinc deficiency (Virgili et al., 1999), there is no information regarding its occurrence during diabetes. In addition there is inadequate information about the small intestinal antioxidant status during diabetes.

The present study was therefore undertaken to determine whether the small intestine is subjected to oxidative damage during diabetes as well as to examine the accompanying changes in antioxidant status in order to understand its role in the pathogenesis of the disease. The nature of regulation of the primary antioxidant enzymes catalase, SOD and GPx was explored by measuring their mRNA levels using RT-PCR. Additionally, the status of the reactive carbonyl compounds detoxifying enzymes, glyoxalases I and II as well as aldose reductase was examined to study the contribution of carbonyl stress, if any, to oxidative damage during diabetes.

2. Materials

Bovine serum albumin (BSA), Tris(hydroxymethyl)aminomethane (Tris), steptozotocin (STZ), pyrogallol, thiobarbituric acid (TBA), 2,4-dinitrophenylhydrazine (DNPH), guanidine hydrochloride, reduced glutathione (GSH), 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), oxidized glutathione (GSSG), nicotinamide adenine dinucleotide reduced phosphate (NADPH), methyl glyoxal, *S*-D-lactoylglutathione, D-glyceraldehyde, nicotinamide adenine dinucleotide (NAD), xanthine, agarose and ethidium bromide were obtained from Sigma (St. Louis, MO, USA). Trizol reagent was purchased from Life Technologies (Gaithersburg, MD, USA) and the one-step RT-PCR kit was purchased from QIAGEN (Germany). All other chemicals used were of the highest analytical grade available.

3. Methods

3.1. Induction of diabetes

Diabetes was induced in male Wistar rats (200–250 g) by a single intraperitonial injection of streptozotocin at a dose of 75 mg/kg body weight in 0.1 M citrate buffer, pH 4.5. Rats showing blood glucose values above 300 mg/dl and persistent glucosuria were selected for the study. Control and diabetic rats were provided food and water ad libitum and maintained for a period of 6 weeks at the end of which, they were weighed, sacrificed and immediately opened surgically and the small intestines were removed and processed.

3.2. Tissue processing

Small intestines were flushed with chilled 1.15% (w/v) KCl solution and the mucosa was scraped. A 10% (w/v) homogenate was prepared in 50 mM phosphate buffer, pH 7.4 and centrifuged at 8000 × *g* for 15 min at 4 °C in a Sorvall RC 5B refrigerated

centrifuge. The supernatant so obtained was used for all the assays. The protein content was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951) using bovine serum albumin as the standard.

3.3. Markers of oxidative stress

3.3.1. Lipid peroxidation

The intestinal homogenate (1 mg protein) was precipitated with trichloroacetic acid (TCA) and reacted with TBA in a boiling water bath for 15 min. After cooling the sample, the absorbance was read at 532 nm and the concentration of malondialdehyde (MDA) was calculated using a molar extinction coefficient value of 153,000 M^{-1} cm^{-1}. The results were expressed as nmol of MDA/mg protein (Esterbauer & Cheeseman, 1990).

3.3.2. Protein carbonyl content

The extent of protein oxidation was determined by measuring the protein carbonyl content. Briefly, the intestinal homogenate (1 mg protein) was incubated with DNPH for 1 h followed by precipitation with TCA. Following centrifugation, the pellet was washed with ethanol–ethyl acetate (1:1) to remove excess DNPH, dissolved in 6 M guanidine hydrochloride and the absorbance measured at 366 nm. The carbonyl content was calculated using a molar extinction coefficient value of 22,000 M^{-1} cm^{-1} and the results expressed as nmol/mg protein (Levine et al., 1990).

3.4. Antioxidant status

Enzymes: (1) Catalase activity was measured according to the method of Aebi (1983) by following the decrease in absorbance of H_2O_2 at 240 nm for 1 min. The enzyme activity was expressed as *k*/sec/mg protein, where *k* is the rate constant of a first-order reaction. (2) Superoxide dismutase activity was measured by the inhibition of pyrogallol autoxidation at 420 nm for 3 min according to the method of Marklund & Marklund (1974). The enzyme activity was expressed as U/mg protein, where 1 U is the amount of enzyme required to bring about 50% inhibition of the autoxidation of pyrogallol. (3) Glutathione peroxidase activity was assayed by the method of Rotruck, Pope, Ganther, Hafner, and Hoekstra (1973) using H_2O_2 as the substrate. Enzyme activity was expressed as μg of glutathione oxidized/min/mg protein. (5) Glutathione reductase activity was measured by following the oxidation of NADPH at 340 nm (Racker, 1955). The extinction coefficient value of 6.2 mM^{-1} cm^{-1} was used for the calculations. Specific activity was expressed as μmol of NADPH oxidized/min/mg protein. (6) Glutathione-*S*-transferase activity was measured by following the formation of the conjugate between GSH and CDNB at 340 nm. The extinction coefficient value of 10 mM^{-1} cm^{-1} was used for calculations. The activity was expressed as μmol/min/mg protein (Awasthi, Dao, & Saneto, 1980).

3.4.1. Total cellular thiols

These were measured as protein and non-protein thiols by the method of Sedlak and Lindsay (1968). Briefly, an aliquot of the homogenate was treated with 6% trichloroacetic acid. Following centrifugation, the supernatant and the pellet dissolved in 2 ml of 0.4 M Tris–HCl buffer containing 10% SDS, were reacted with DTNB and the absorbance was measured at 412 nm. A calibration curve was obtained using reduced glutathione as standard and the protein and non-protein thiol contents were expressed as nmol/mg protein.

3.5. Reactive carbonyl compounds detoxifying enzymes

(1) Glyoxalase I was measured by following the formation of *S*-D-lactoylglutathione at 240 nm. The extinction coefficient value of 3.1 mM^{-1} cm^{-1} was used for calculations. Specific activity was expressed as μmol/min/mg protein (Ratliff, Vander Jagt, Eaton, & Vander Jagt, 1996). (2) Glyoxalase II activity was measured by following the hydrolysis of *S*-D-lactoylglutathione at 240 nm. The extinction coefficient value of 3.1 mM^{-1} cm^{-1} was used for the calculations. Specific activity was expressed as μmol/min/mg protein (Ratliff et al., 1996). (3) Aldose reductase activity was determined by monitoring the oxidation of NADPH at 340 nm using D-glyceraldehyde as the substrate. The extinction coefficient value of 6.2 mM^{-1} cm^{-1} was used for calculations. Specific activity was expressed as μmol/min/mg protein (Ratliff et al., 1996).

3.6. Xanthine oxidase and xanthine dehydrogenase activities

These were measured by following the production of uric acid at 295 nm. The assay was carried out in the presence of 0.4 mM NAD to measure xanthine oxidase (XO) and xanthine dehydrogenase (XDH) and in the absence of NAD to measure xanthine oxidase only. The molar extinction coefficient value of uric acid, 12,200 M^{-1} cm^{-1} was used for calculations. The enzyme activity is expressed as U/mg protein, where 1 U corresponds to 1 μmol of urate formed per minute at 25 °C (Parks, Williams, & Beckman, 1988).

3.7. RT-PCR of antioxidant enzymes

This was performed using the one-step RT-PCR kit (QIAGEN, Germany) with 1 μg of total RNA isolated from freshly scraped mucosa by the method of Chomzynski (1993) and 0.6 μM of the forward and reverse gene specific primers (Table 1) in a thermal cycler (Techne, Cambridge, UK). β-Actin gene primers were used as house-keeping control. The program was optimized to obtain linearity between the amount of template used and the amount of product formed. Reverse transcription and amplification of all the genes of interest were done simultaneously as follows: (1) reverse transcription—50 °C, 30 min; (2) initial PCR activation—95 °C, 15 min; (3) three-step cycling for 30 cycles involving (a) denaturation—94 °C, 30 s, (b) annealing—58 °C, 30 s and (c) extension—72 °C, 1 min. The PCR products were resolved on agarose (1.5%) gels, stained with ethidium bromide and were photographed using a Polaroid camera. The images were digitized using a scanner and quantification done using the Scion Imaging software provided by the Scion Corporation (Maryland, USA) (http://www.scioncorp.com).

3.8. Statistical analysis

Data are expressed as mean ± S.D. Statistical differences were determined by the Student's *t*-test for unpaired observations and were considered significant if *P*-values were less than 0.05.

4. Results

At the end of 6 weeks, the intestines of the diabetic rats were significantly longer and the mucosal yield higher compared to controls. Significant increases in both lipid peroxidation and the protein carbonyl content confirm the occurrence of oxidative damage in diabetic rats. The increase in lipid peroxidation (four-fold) was greater than the increase (38%) in protein carbonyl content (Table 2). A reduction in the activities of XO and XDH by 25.7 and 42.6% (Table 3) indicate that these enzymes are not likely to be the source of ROS. The total cellular thiols of the intestinal epithelial cells, measured in terms of protein thiols (20.75 ± 7 nmol/mg protein, $n = 5$) and non-protein thiols (1.42 ± 0.37 nmol/mg protein, $n = 5$) were found to be unaltered in diabetic rats. Further the activities of the enzymes of the glutathione antioxidant system, i.e. GR (0.075 ± 0.016 μmol/min/mg protein, $n = 5$) and GST (0.01 ± 0.005 μmol/min/mg protein, $n = 5$) were also unchanged by diabetes. The activities of glyoxalases I and II as well as aldose reductase, enzymes of the reactive carbonyl compound detoxification machinery were also unaltered at the end of 6 weeks of diabetes (Table 3). In contrast,

Table 1
Sequences of the PCR primers and the product sizes of the genes amplified

Gene	Genbank accession no.	Forward primer	Reverse primer	Size of the PCR product (bp)
β-Actin	V01217	5′-CCTGCTTGCTGATCCACA	5′-CTGACCGAGCGTGGCTAC	505
CuZn superoxide dismutase	X05634	5′-GCAGAAGGCAAGCGGTGAAC	5′-TCACACCACAAGCCAAGCGG	387
Glutathione peroxidase	M21210	5′-CTCTCCGCGGTGGCACAGT	5′-CCACCACCGGGTCGGACATAC	290
Catalase	AH004967	5′-GCGAATGGAGAGGCAGTGTAC	5′-GAGTGACGTTGTCTTCATTAGCACTG	670

Table 2
Effect of diabetes on the activities of the antioxidant enzymes and the levels of lipid peroxidation and protein carbonyls of the intestinal mucosa

	Control	Diabetic
Catalase *k*/sec/mg protein	0.025 ± 0.001	0.057 ± 0.007*
Superoxide dismutase (U/mg protein)	7.538 ± 1.050	12.956 ± 1.370*
Glutathione peroxidase (μg of glutathione utilized/min/mg protein)	2.894 ± 0.584	0.934 ± 0.208*
Lipid peroxidation (nmol MDA/mg protein)	0.174 ± 0.06	0.719 ± 0.08*
Protein carbonyl content (nmol/mg protein)	0.932 ± 0.021	1.286 ± 0.036*

Values are expressed as mean ± S.D. ($n = 6$).
* Significantly different from control at $P < 0.001$.

Table 3
Effect of diabetes on the activities of xanthine oxidase, xanthine dehydrogenase and the enzymes detoxifying reactive carbonyl compounds

	Control	Diabetic
Xanthine oxidase (U/mg protein)	3.715 ± 0.206	2.759 ± 0.529**
Xanthine dehydrogenase (U/mg protein)	2.888 ± 0.263	1.656 ± 0.189*
Glyoxalase I (μmol/min/mg protein)	0.258 ± 0.053	0.289 ± 0.051
Glyoxalase II (μmol/min/mg protein)	0.036 ± 0.006	0.037 ± 0.006
Aldose reductase (μmol/min/mg protein)	0.008 ± 0.002	0.010 ± 0.005

Values are expressed as mean ± S.D. ($n = 5$).
* Significantly different from control at $P < 0.001$.
** Significantly different from control at $P < 0.01$.

the activities of the primary antioxidant enzymes, catalase, SOD and GPx were subjected to significant alterations during this period. While catalase activity was increased by 123.9% and SOD by 71.8%, that of GPx decreased by 67.7% (Table 2). No significant differences in the steady state mRNA levels of these enzymes were observed between control and diabetic groups (Fig. 1).

5. Discussion

The results of our study demonstrate the occurrence of oxidative damage in the small intestine during experimental diabetes. It is likely to be both the cause and consequence of some of the associated changes in intestinal function. The observed increases in lipid peroxidation levels in the small intestine are in agreement with similar findings in other tissues (Kakkar et al., 1995). Lipid peroxidation may bring about protein damage and inactivation of membrane bound enzymes either through direct attack by free radicals or through chemical modification by its end products, malondialdehyde and 4-hydroxynonenal (Halliwell & Gutteridge, 1999). It is also known to decrease the fluidity of the intestinal brush border membrane (Ohyashiki, Ohtsuka, & Mohri, 1986). Therefore the observed increase in lipid peroxidation could provide an additional explanation for the previously reported decrease in fluidity of the intestinal brush border membrane during diabetes attributed to changes in lipid composition alone (Brasitus & Dudeja, 1985).

The diabetes-induced stimulation of intestinal mucosal growth is believed to be a response to elevated physiological demands. The accompanying increase in the transport of oxidizable compounds such as glucose (Fedorak, 1990), amino acids (Fedorak, 1990), lipids (Staprans, Rapp, Pan, & Feingold, 1993) along with the increased synthesis of cholesterol (Feingold et al., 1982) and triglycerides (Feingold et al., 1990) and decreased utilization of glucose within the enterocyte (Madsen, Ariano, & Fedorak, 1995) can lead to transient increases in the intracellular concentrations of these compounds. The free radicals generated by autoxidation of these compounds may have been responsible for the elevation in lipid peroxidation and protein oxidation. In addition, glucose is known to induce lipid peroxidation through activation of the

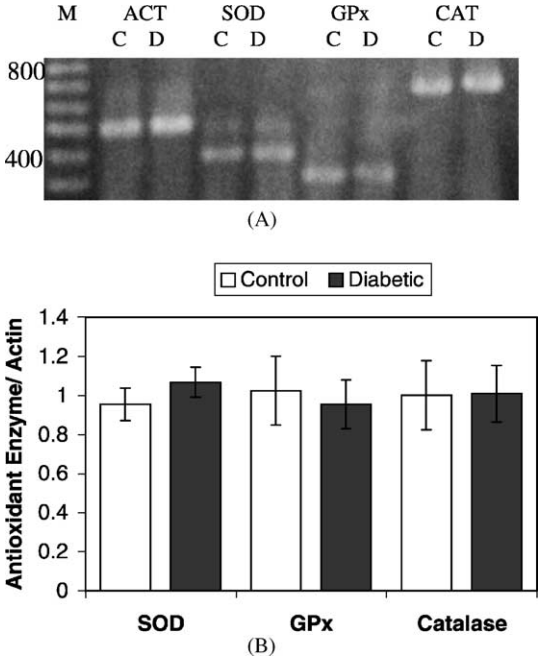

Fig. 1. The mRNA expression of the antioxidant enzymes, CuZn-SOD, GPx and catalase in the small intestinal mucosa of control and diabetic rats was studied by RT-PCR. Each total RNA preparation (1 μg) was reverse transcribed and the cDNA products were amplified by PCR (30 cycles) using gene specific primers. (A) Agarose gel (1.5%) electrophoresis of the PCR products. The numbers indicate the band sizes of the DNA ladder. The bands correspond to β-Actin (ACT), SOD, GPx and catalase from control and diabetic rats. (B) The ratios of CuZnSOD, GPx and catalase to β-Actin mRNA in control and diabetic rats. Values are expressed as mean ± S.D. ($n = 5$) No significant differences were found.

lipoxygenase enzymes (Rajeswari, Natarajan, Nadler, Kumar, & Kalra, 1991).

One of the probable sources of increased ROS in the intestinal mucosa is the enzyme xanthine oxidase (Parks et al., 1988). Increased XO activity has been linked to intestinal injury in ischaemia/reperfusion (Halliwell & Gutteridge, 1999) and surgical stress (Prabhu et al., 2000). Additionally XO has been implicated in free radical production during diabetes based on the observed decrease in oxidative stress in diabetic patients during treatment with the XO inhibitor, allopurinol (Desco et al., 2002). The decrease in XO activity (Table 3) observed in this study is consistent with similar findings in liver and kidneys of diabetic rats (Satav, Dave, & Katyare, 2000) and rules out its involvement in raising the levels of ROS. Though the physiological significance of the decreases in XO and XDH activities is unclear, these are likely to be the result of alterations in purine catabolism and may consequently lead to lowering of the levels of the antioxidant, uric acid.

The altered balance of the antioxidant enzymes caused by an increase in catalase and SOD and the decline in GPx activities (Table 2) may be responsible for the inadequacy of the antioxidant defenses in combating ROS-mediated damage. The increased activities of catalase and SOD may be a response to increased production of H_2O_2 and $O_2^{-\bullet}$ by the autoxidation of glucose and non-enzymatic glycation. In contrast to the observed increase in SOD activity, a decrease has been reported after 9 days of diabetes (Loven, Schedl, Oberley, Wilson, & Neihaus, 1982). This is in corroboration with the reported time dependant changes in the activities of the antioxidant enzymes during diabetes (Kakkar, Mantha, Radhi, Prasad, & Kalra, 1997). Giron et al. (1999) have reported a lack of change in the activities of intestinal catalase, SOD and GPx of diabetic rats fed diets containing different fat supplements. Diet-induced decrease in glucose transport (Thomson et al., 1987) may influence the level of free radical generation and oxidative damage. This may probably be the reason for the differences in the response of the antioxidant enzymes to diabetes between the present study and that of Giron et al. (1999).

The opposing responses of catalase and GPx (Table 2), both of which breakdown H_2O_2, are in agreement with those reported in kidneys of diabetic rats (Kakkar et al., 1997). These suggest the existence of compensatory mechanisms in response to increased oxidative stress such that tissues lacking one of the above enzymes may be critically dependant on the other. However, the increase in catalase activity may be insufficient to tackle the increased H_2O_2 production contributing to further lipid peroxidation through generation of hydroxyl radicals by the Fenton reaction (Halliwell & Gutteridge, 1999). The decrease in GPx activity correlates with the increase in lipid peroxidation (Table 2). This may account for the increased levels of oxidized lipids in the serum lipoproteins of diabetic rats following consumption of a diet rich in oxidized lipids (Staprans et al., 1993) since the intestinal GPx detoxifies dietary lipids before they enter the circulation (Halliwell et al., 2000).

To our knowledge, this is the first investigation of the small intestinal antioxidant enzyme expression during diabetes. The unaltered mRNA levels for all the genes tested (Fig. 1) clearly shows, that the observed changes in the activities of catalase, SOD and GPx are not due to altered transcription. There are similar reports of alterations in catalase and SOD activities without corresponding changes in mRNA expression, under non-diabetic conditions (Clerch & Massaro, 1992; Virgili et al., 1999). For instance, elevated catalase activity in the lungs of newborn rats exposed to hyperoxia, has been attributed to an increase in mRNA stabilization rather than an increase in transcription (Clerch & Massaro, 1992). Also, an increase in intestinal CuZnSOD activity without a corresponding upregulation of gene expression was observed in rats subjected to zinc deficiency (Virgili et al., 1999). A reduction in the rate of degradation of the enzyme proteins may also lead to the increased activities of catalase and SOD. The decrease in GPx activity despite unaltered mRNA level could be due to inactivation by $O_2^{-\bullet}$ anion (Blum & Fridovich, 1985) or post-translational modification such as non-enzymatic glycation of the enzyme protein (Baldwin, Lee, Leung, Muruganandam, & Mutus, 1995). Though a decrease in the rate of mucosal protein translation caused by insulin deficiency (Charlton, Ahlman, & Nair, 2000) may also result in a specific decrease in GPx activity, several enzyme activities are known to increase during diabetes (Sharma & Sivakami, 1998). Therefore a decrease may be the result of regulation of specific mRNA species.

The absence of changes in the activities of the enzymes of the GSH antioxidant system, GR and GST and the levels of cellular thiols in the intestine in contrast to the alterations in the activities of the primary antioxidant enzymes indicate an attempt to maintain the cellular GSH metabolism probably and minimise the consequences of oxidative stress. The observation of unaltered intestinal non-protein thiol, i.e. GSH content in the present study is in agreement with an earlier report (Loven et al., 1986). It could be the result of restoration of GSH levels depleted due to oxidative stress, by increased synthesis, uptake from the circulation (Mak, Ip, Li, Poon, & Ko, 1996) or luminal contents or regeneration from protein mixed disulfides (Halliwell & Gutteridge, 1999). The decreased utilization of GSH due to the fall in GPx activity may also compensate for the loss of GSH due to oxidation and contribute towards maintaining the cellular GSH levels.

The increase in protein carbonyl content is indicative of both oxidative damage as well as chemical modification. The absence of any changes in the activities of glyoxalases I and II and aldose reductase, which detoxify reactive carbonyls like methylglyoxal, may be responsible for the increase in intestinal protein carbonyl content of diabetic rats in the present investigation. The resulting carbonyl stress may also have contributed to oxidative stress. The possibility of increased chemical modification of the intestinal epithelial cell contents is also suggested by reports of delayed bacterial clearance and consequent overgrowth in the intestinal lumen during diabetes (Bouchier & Hodgson, 1993) that is likely to contribute to increased levels of methylglyoxal through the action of bacterial methylglyoxal synthase.

In conclusion, the results of the study demonstrate the occurrence of oxidative stress in the small intestine during diabetes. Carbonyl stress and the inadequacy of the antioxidant defenses are the probable sources leading to it. Post-translational modification rather than transcriptional changes could be responsible for the alterations in the antioxidant enzyme activities. The reported increases in the functions of some of the brush border membrane proteins such as the sugar and amino acid transporters (Fedorak, 1990) and the glycosidases (Sharma & Sivakami, 1998), in spite of oxidative damage are intriguing. Recently, suppression in apoptosis of intestinal epithelial cells during diabetes has been shown (Noda et al., 2001). This can result in a decline in the rate of disposal of oxidatively damaged epithelial cells further aggravating the damage. Since the small intestine is a primary site for the action of dietary antioxidants, their ability to prevent diabetes-induced oxidative damage to the small intestine may be significant in overcoming oxidative stress.

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