Antioxidant Enzymes in Rat Gastrointestinal Tract

M MANOHAR & K A BALASUBRAMANIAN
The Wellcome Research Unit, Christian Medical College Hospital, Vellore 632 004
Antioxidant Enzymes in Rat Gastrointestinal Tract

M ANGHER & K A BALASUBRAMANIAN

The Wellcome Research Unit, Christian Medical College Hospital, Vellore 632 004

Received 3 February 1986; revised 11 June 1986

The enzymes responsible for defence of the cell against oxidant injury are superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and catalase. The distribution of these enzymes is studied in various regions of the gastrointestinal tract of rat, in villus and crypt cells of small intestine and in its subcellular fractions. The specific activity of glutathione peroxidase and CuZn-superoxide dismutase is maximal in the stomach. Catalase activity is uniform in all regions of the gastrointestinal tract, whereas glutathione-S-transferase activity is high in the small intestine. Villus cells in the small intestine have more glutathione peroxidase, glutathione-S-transferase and superoxide dismutase activities as compared to crypt cells. Among subcellular fractions studied cytosol has the maximum activity of all these enzymes except Mn-superoxide dismutase which is mainly associated with mitochondrial fractions. Age-dependent distribution studies show that the specific activity of glutathione peroxidase and catalase is uniform from weaning to adult rat and a gradual increase in the specific activity of superoxide dismutase and glutathione-S-transferase is observed with development.

Free radicals damage cells by initiating lipid peroxidation of the membrane lipids. This oxidative damage is mainly due to activated oxygen species such as superoxide radical, hydroxy radicals and singlet oxygen. Cells detoxify these activated oxygen species by their defence enzymes superoxide dismutases, catalase and glutathione peroxidases. Superoxide dismutases rapidly accelerate the dismutation reactions of superoxide radicals resulting in the formation of hydrogen peroxide, which is removed by the action of catalase and glutathione peroxidases. Selenium and non-selenium glutathione peroxidase reduce potentially harmful hydroperoxy fatty acids to harmless hydroxy lipids. The role of these defence enzymes has been studied in lung, brain, pancreatic islets and eye lens.

Gastrointestinal tract is more vulnerable to peroxidative damage since mucosa is constantly exposed to dietary lipids, drugs, variety of proxidants like ascobic acid and transition metals and all of them can induce lipid peroxidation. Iron is present in free from in the lumen of the gut which can induce considerable amount of lipid peroxidation. Lumen of the gut is rich in various types of bacteria and their metabolism may result in the production of a variety of proxidants. In fat malabsorption, such as seen in tropical sprue, lipids accumulate inside the absorptive cells. The presence of antioxidant tocopherol and other defence enzymes can give protection to these cells. We have earlier shown that the tocopherol content of the gastrointestinal tract is similar to other tissues. Reddy and Tappel have studied the effect of peroxidised oil and selenium on the glutathione peroxidase of gastrointestinal tract and Lane et al. studied the effect of various levels of selenium, sulfur and α-tocopherol on glutathione peroxidase activity in intestine and liver. Apart from this, very little information is available on these defence enzymes in the gastrointestinal tract. In the present work we have studied the distribution of these enzymes along the gastrointestinal tract, their activity in villus and crypt cells, subcellular localisation and developmental pattern with age. Since glutathione-S-transferase has also been implicated in the defence of the cell against peroxidation damage, this enzyme is also included in this study.

Materials and Methods

Dithiothreitol, DNA, p-nitrophenylphosphate, cytochrome C, glucose-6-phosphate, NADH, NADPH and nitroblue tetrazolium were obtained from Sigma Chemical Co. USA. GSH was obtained from Boehringer, Germany. Cumene hydroperoxide was from Koch-Light Laboratories, England. All other reagents used were of analytical grade.

Tissue preparations — Adult (Wistar) rats were fasted for 24 hr (water given ad libitum) and killed by decapitation. Small intestine was excised, washed with ice cold 1.15 % KCl and the mucosa was obtained by opening intestine longitudinally and scraping. For the studies of developmental pattern of enzymes, rats of different ages were killed and small intestines were excised and washed with ice cold 1.15 % KCl. For regional distribution study stomach, duodenum, jejunum, ileum, colon and rectum were excised separately, washed and mucosa collected.

Separation of villus and crypt cells — Villus and crypt cells in small intestine were separated according to the method of Weiser. In principal, the epithelial cells...
were released in a sequential manner from the villus tip to the crypt base by repeated incubations with phosphate buffered saline containing EDTA and dithiothreitol.

Subcellular fractionation—Subcellular fractionation of the intestinal mucosa was carried out according to the procedure of Hubscher.\textsuperscript{24} Intestinal mucosal homogenate (10%) was prepared in 0.3 M sucrose, (pH 7.4) using Teflon-glass homogeniser, centrifuged successively at 1500 g for 10 min, 12000 g for 15 min and 105000 g for 60 min to pellet nuclear, mitochondrial and microsomal fractions and each fraction was suspended in 0.3 M sucrose pH 7.4 and used for the studies. Purity of the subcellular fractions was judged using the markers namely DNA, alkaline phosphatase, cytochrome oxidase, glucose-6-phosphatase and lactate dehydrogenase.

Measurement of enzyme activities—Protein was determined by Lowry’s procedure.\textsuperscript{26} DNA was estimated using thionbarbituric acid.\textsuperscript{27} Alkaline phosphatase activity was assayed using p-nitrophenyl phosphate as substrate.\textsuperscript{28} Cytochrome oxidase assay was done as described by Cooperstein.\textsuperscript{29} Activity of glucose-6-phosphatase was determined as described by Hubscher.\textsuperscript{30} Lactate dehydrogenase activity was determined spectrophotometrically using NADH.\textsuperscript{31} In subcellular fractions and homogenate defence enzymes were assayed after sonication for 3 min in burst of 30 seconds with cooling at 0°C. Glutathione peroxidase activity was determined spectrophotometrically by the method of Nakamura.\textsuperscript{25} Hydrogen peroxide (0.25 mM) and cumene hydroperoxide (1.25 mM) were used as substrate to differentiate Se-glutathione peroxidase from non-Se-glutathione peroxidase activity.\textsuperscript{33} One unit of glutathione peroxidase corresponds to oxidation of 1 μmole of NADPH per minute. Catalase activity was measured spectrophotometrically by following the break down of hydrogen peroxide.\textsuperscript{34} Activity that brought about a disproportionation of hydrogen peroxide at a rate of \(10^{-5}\) optical density per second, which was measured at abs. max 240 was considered as one unit. Superoxide dismutase was assayed according to the method of Beauchamp and 1.25 mKCN was used in this assay to differentiate the CN\textsuperscript{-} sensitive CuZn-superoxide dismutase from CN\textsuperscript{-} insensitive Mn superoxide dismutase.\textsuperscript{35} One unit of superoxide dismutase is the activity that produce 50\% inhibition in nitroblue tetrazolium colour production. Glutathione-s-transferase activity was measured using 1-chloro-2,4-dinitrobenzene as substrate. One unit of activity is one micromole of substrate utilised per minute at 25°C.\textsuperscript{36}

Electrophoresis—Disc gel electrophoresis was done in 7% polyacrylamide gels\textsuperscript{37} and the superoxide dismutase activity was localised as described.\textsuperscript{38}

Results

Table 1 shows the regional distribution of the defence enzymes in the gastrointestinal tract. Stomach has more activity of CuZn-superoxide dismutase and of non-Se-glutathione peroxidase than rest of the gastrointestinal tract. The activity of Se-glutathione peroxidase is maximum in ileum and colon and minimum in jejunum and duodenum. Mucosa of rectum, colon, duodenum and ileum have similar CuZn-superoxide dismutase activity and jejunal mucosa shows minimal activity. Mn-superoxide dismutase activity in all regions of the gut is lower than CuZn-superoxide dismutase. Catalase exhibits similar activity in all regions of the gastrointestinal tract except in ileum and colon which possess higher activity. Ileum has the highest activity of glutathione-s-transferase followed by duodenum and jejunum and other regions have less activity of this enzyme.

Separated villus and crypt cells are comparatively pure as judged by the marker enzyme alkaline phosphatase and by electronmicroscopy. Se- and non Se-glutathione peroxidase and glutathione-s-

<table>
<thead>
<tr>
<th>Region</th>
<th>Se-glutathione peroxidase</th>
<th>Non-Se glutathione Peroxidase</th>
<th>Catalase</th>
<th>CuZn superoxide dismutase</th>
<th>Mn superoxide dismutase</th>
<th>Glutathione s-transferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>35.2 ± 3.6</td>
<td>58.8 ± 6.0</td>
<td>2.42 ± 0.6</td>
<td>32.72 ± 0.4</td>
<td>6.57 ± 0.6</td>
<td>0.44 ± 0.1</td>
</tr>
<tr>
<td>Duodenum</td>
<td>10.55 ± 2.4</td>
<td>7.29 ± 0.8</td>
<td>2.42 ± 0.8</td>
<td>11.87 ± 0.4</td>
<td>3.05 ± 0.1</td>
<td>1.69 ± 0.1</td>
</tr>
<tr>
<td>Jejunum</td>
<td>17.39 ± 0.6</td>
<td>6.30 ± 0.1</td>
<td>1.60 ± 0.1</td>
<td>3.78 ± 0.3</td>
<td>2.99 ± 0.7</td>
<td>1.40 ± 0.5</td>
</tr>
<tr>
<td>Ileum</td>
<td>33.41 ± 6.2</td>
<td>5.48 ± 0.1</td>
<td>4.95 ± 0.7</td>
<td>9.08 ± 0.4</td>
<td>5.59 ± 0.3</td>
<td>3.58 ± 0.2</td>
</tr>
<tr>
<td>Colon</td>
<td>80.30 ± 14.2</td>
<td>6.36 ± 5.0</td>
<td>3.98 ± 1.2</td>
<td>12.99 ± 1.8</td>
<td>ND</td>
<td>0.47 ± 0.2</td>
</tr>
<tr>
<td>Rectum</td>
<td>ND</td>
<td>ND</td>
<td>1.75 ± 0.6</td>
<td>13.22 ± 1.4</td>
<td>1.94 ± 0.3</td>
<td>0.35 ± 0.1</td>
</tr>
</tbody>
</table>

ND = Not Detectable
transferase activities are maximum in villus cells and gradually decrease towards crypt cells (Fig. 1). Similarly, activities of CuZn- and Mn-superoxide dismutase are also higher in villus cells than in the crypt cells. Mn-superoxide dismutase activity is almost absent in the crypt cells. Catalase activity is unevenly distributed in villus and crypt cells.

The purity of the subcellular fractions of intestinal mucosa was judged by the marker enzymes (Fig. 2). Among various subcellular fractions of intestine, cytosol contains the maximum activities of Se- and non-Se-glutathione peroxidase, glutathione-s-transferase, catalase and CuZn-superoxide dismutase (Fig. 2). Mn-superoxide dismutase activity is highest in mitochondria followed by cytosol fraction. All the enzyme activities are minimal in nuclear and brush border membrane fractions and 15-20% of the activities of Se- and non-Se-glutathione peroxidase are present in mitochondrial as well as microsomal fractions. Microsomal fraction shows very low activities of CuZn- and Mn-superoxide dismutase.

Studies on the developmental pattern of these enzymes in the intestinal mucosa show that glutathione peroxidase and catalase activities are not varied during development whereas there is a gradual increase in the specific activities of glutathione-s-transferase, CuZn- and Mn-superoxide dismutase with increase in age. Newborn rat intestine has very low superoxide dismutase activity which increases gradually with age and reaches maximum in the adult rat (Fig. 3). Appearance of the two electrophoretically distinct CuZn-superoxide dismutase activities can also be observed during development (Figure not shown).

Discussion

Peroxidation of membrane lipids leads to cell necrosis and death and protection to this is offered by the defence enzymes such as glutathione peroxidases, catalase, superoxide dismutases and the membrane tocopherol. Mucosa of the gastrointestinal tract is more vulnerable to peroxidative damage since it is in constant contact with various oxidants from diet and bacterial metabolites. There are evidences for the production of free radicals in intestine during ischemic condition. Addition of xanthine to whole intestinal cells results in the production of free radicals by the activation of indoleamine dioxygenase activity. Hence, the study of the defence system
against peroxidation in the gastrointestinal tract assumes more importance.

Regional distribution studies show that stomach has the highest activity of glutathione peroxidases and superoxide dismutases. In the gastrointestinal tract stomach is the first organ exposed to dietary materials and hence it is important to have sufficient activities of antioxidant enzymes. The work by Reddy and Tappel has shown that the glutathione peroxidase activity can be induced in the stomach by feeding peroxidised oil. When compared with catalase, glutathione peroxidase can act on low concentration of hydrogen peroxides in addition to other organic peroxides and it may offer a better protection for the cells lining the stomach from peroxidation.

The villus cells of the intestine are directly exposed to dietary materials and the probability of oxidant damage to these cells is comparatively higher than the crypt cells. Activity measurements show that villus cells contain higher activity of glutathione peroxidases and superoxide dismutases as compared to crypt cells. Due to interference in the assay of superoxide dismutase, the activity in the first fraction of villus region could not be detected. It has been shown that crypt cells have more glutathione as compared to villus cells and this may be due to the presence of various glutathione utilising enzymes in the villus cells. Studies on subcellular fractions show that most of the antioxidant enzymes are present in the cytosol. Aerobic metabolism results in the production of free radicals like superoxide anion and hydroxyl radicals, which in turn produce toxic lipid peroxides. This may be metabolised by the cytosolic defense enzymes, so that the membrane damage by these peroxides will be considerably reduced. Due to oxidative metabolisation, the possibility of production of free radicals in mitochondria is relatively high and the presence of Se- and non-Se-glutathione peroxidase and Mn-superoxide dismutase in mitochondria may offer protection. Microsomes are normally involved with hydroxylation, which sometimes can oxidise unsaturated lipids of the endoplasmic reticulum, and produce lipid peroxides. This peroxidation may be inhibited by microsomal glutathione peroxidase. Intestinal epithelial cell membranes also possess tocopherol which serves as an antioxidant. The location of antioxidant tocopherol in the membrane and the defence enzymes in the cytosol and in other subcellular fractions of mucosal cells may be important to defend the cell against oxidant injury by free radicals.

The specific activity of glutathione peroxidase and catalase is unchanged with age whereas that of superoxide dismutase increases with age. The developmental pattern of these enzymes cannot be correlated with each other because the expression of these enzymes may differ. Early development of the activity of glutathione peroxidase and catalase may be sufficient enough to protect the gastrointestinal tract against oxidant injury. The same pattern has also been observed in the developing rat brain.

Glutathione-s-transferase, besides conjugating GSH with xenobiotics protects cellular membranes from oxidative stress by expressing selenium-independent glutathione peroxidase activity. The activity of this enzyme is high in small intestine and is mainly localised in the cytosol and this may aid other antioxidant enzymes in removing toxic substances before damaging the membrane. Like superoxide dismutase the specific activity of glutathione-s-transferase also increases with age.

The combined action of superoxide dismutase,
glutathione peroxidase and catalase provides an efficient mechanism for the removal of free radicals from the cells. Deficiency of these activities may result in cell death and organ necrosis. For instance Sc- 
deficiency lowers the level of glutathione peroxidase, allows the accumulation of hydrogen peroxides or organic peroxides in the cells, initiates lipid peroxidation and results in the loss of membrane integrity. Supplementation of diet with peroxided oil was found to induce glutathione peroxidase in various tissues of rat. Recently Cross et al have postulated that the mucus layer of the gastrointestinal tract might give a protection from the peroxidative damage. This present study has shown the presence of defence enzymes in the gastrointestinal tract and these enzymes along with the membrane antioxidant tocopherol may provide a better protection against oxidant injury.

Acknowledgement

The authors thank Prof. V.1. Mathan, Head of this Unit for his continued interest in this work and Mr. S. Moses Narendran for his secretarial assistance. The Wellcome Research Unit is supported by the Wellcome Trust, London.

References

42. Cornell J S & Meister (1976), Proc Nail Acad Sci USA, 73, 420.