

Low Glutathione and Glutathione S-Transferase Levels in Barrett's Esophagus as Compared to Normal Esophageal Epithelium

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Patients with Barrett's esophagus, wherein squamous epithelium has been replaced by columnar epithelium, have an increased risk for developing esophageal adenocarcinoma as compared to the general population. Glutathione S-transferase (GST), a family of detoxification enzymes consisting of class α , μ , π , and θ isoforms, is involved in detoxification of carcinogens and low levels of these enzymes correlated with high cancer risk. We have now compared GST enzyme activity, GST isoenzyme composition and glutathione (GSH) content of Barrett's mucosa with that of adjacent normal squamous epithelium. Biopsy specimens of 98 patients with Barrett's esophagus were taken from both Barrett's and adjacent normal squamous epithelium. GST enzyme activity towards 1-chloro-2,4-dinitrobenzene was measured, and GST isoenzyme levels were determined by densitometrical analyses of western blots after immunodetection with monoclonal antibodies. Total GSH content was determined by high-performance liquid chromatography after conjugation with monobromobimane. Wilcoxon's signed rank test and Spearman correlation analyses were used for statistical evaluation. As compared with adjacent normal squamous epithelium, GST enzyme activity in Barrett's epithelium was reduced by 35%, and GST μ , GST π and GSH levels were reduced by 24%, 30%, and 63%, respectively. However, the minor GST α and GST θ levels were higher in Barrett's epithelium (by 625% and 33%, respectively). High levels of GSH and GSTs in general are correlated with protection against cellular or cytogenetic damage. The observed reduction in GSTs and GSH in Barrett's epithelium may therefore contribute to the increased cancer risk in this tissue.

Key words: Barrett's esophagus — Glutathione — Glutathione S-transferase — Metaplasia

Barrett's esophagus is a pathological condition in which stratified squamous epithelium has been replaced by columnar epithelium in the lower part of the esophagus.¹⁻⁴ This condition was first described by Dr. Norman Barrett in 1950.³ Three types of columnar epithelium have been identified: gastric-cardiac or junctional type epithelium; gastric-fundic epithelium; and a distinctive type of specialized epithelium with the villous surface lined by columnar epithelium and goblet cells.⁴ Compared to the general population, patients with Barrett's esophagus have a 30- to 125-fold increased risk of developing esophageal adenocarcinoma.⁵⁻⁹ A 10% yearly rate of increase of adenocarcinomas of the esophagus and gastric cardia was found in males, which exceeds that of any other cancers.¹⁰

A large proportion of human cancers are claimed to be caused by lifestyle or dietary factors.¹¹ Our diet contains many toxic or potentially carcinogenic compounds which

are absorbed and metabolized in the gastrointestinal tract. Metabolizing or biotransformation enzymes such as glutathione S-transferases (GSTs) are present in the epithelial cells along the human gastrointestinal tract.¹²⁻¹⁴ Human cytosolic GSTs are a family of dimeric enzymes, divided into the main classes α , μ , π and θ .¹⁵⁻¹⁸ GSTs catalyze the binding of a large variety of electrophiles to the sulfhydryl group of glutathione (GSH), yielding less harmful and more water-soluble molecules, which can be excreted via bile or urine. Since the most reactive, ultimate carcinogenic forms of chemicals are generally electrophiles, GST takes considerable importance as a mechanism for carcinogen detoxification.^{15,16} Recently, a significant negative correlation was demonstrated between GST enzyme activity in the mucosa along the gastrointestinal tract and the tumor incidence.¹³ Deficiencies of GST μ and/or GST θ have been claimed to increase the risk of developing gastric or colonic cancer,¹⁹ or lung cancer in smokers,²⁰ although other studies deny such a genetic predisposition.²¹ However, low or reduced levels of GSH and GSTs

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may result in a lower capacity to detoxify carcinogens, which may result in more cytogenetic damage and account for an increased tumor risk.

We have studied the GSH and GST isoenzyme levels in Barrett's epithelium and compared them with the levels in adjacent normal squamous esophageal epithelium.

MATERIALS AND METHODS

Tissue Tissue samples of 98 patients with Barrett's esophagus (mean age 61 ± 2 year; range 25–93) were obtained during routine endoscopic inspection. Three biopsies were obtained from the Barrett's epithelium and three biopsies were also taken from the adjacent squamous epithelium. Biopsies were frozen in liquid nitrogen immediately, and were stored at -80°C until use. Barrett's epithelium in the lower esophagus was confirmed in all patients after investigation of biopsy specimens by a pathologist. Barrett's epithelium was of the intestinal, gastric-fundic and junctional type in 95, 2 and 1 cases, respectively. In 48 samples of Barrett's epithelium, no dysplastic reaction was evident, whereas mild, moderate and severe dysplasia was found in 22, 20 and 8 patients, respectively. Tissue was homogenized in an Eppendorf tube using a plastic pestle, after dilution in approximately 6 volumes of 20 mM Tris/HCl buffer pH 7.4, containing 0.25 M sucrose and 1.4 mM dithiothreitol. Cytosolic fractions were made by centrifugation at $150,000g$ for 50 min. The investigations were approved by the local ethical committee on human experimentation.

Biochemical assays Protein was assayed in triplicate by the method of Lowry *et al.*,²²⁾ using bovine serum albumin as a standard. Total GST enzyme activity was determined in triplicate according to Habig *et al.*,²³⁾ using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. Specific GST isoenzyme levels were determined as described before.²⁴⁾ In short, cytosolic fractions were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (11% acrylamide, w/v), and subsequently to western blotting, using a semidry blotting system (Novablot II, Pharmacia, Uppsala, Sweden). Western blots were incubated with monoclonal antibodies against human GST class α , μ , π and θ . Class α antibodies react with human GST A1-1, GST A1-2 and GST A2-2.²⁴⁾ Class μ antibodies recognize human GST M1a-1a, GST M1a-1b and GST M1b-1b.^{25, 26)} Class π antibodies react with human GST P1-1.²⁴⁾ Class θ antibodies react with GST T1-1.²⁷⁾ Typical examples of results on western blots obtained with these antibodies are shown in refs. 24–27. The specific binding of the monoclonal antibodies to the isoenzymes was detected by incubation with peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts, Glostrup, Denmark) and subsequent development of the peroxidase label with 4-chloro-1-naphthol and hydrogen peroxide (for GST α , μ

and π). Staining of GST θ was performed in 0.1% 3,3'-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline (PBS) containing 0.01% hydrogen peroxide (Merck, Darmstadt, Germany), 0.34 g/liter imidazole and 0.26 g/liter cobalt chloride- $6\text{H}_2\text{O}$. Staining intensity on the immunoblots was quantified using a laser densitometer (Ultrosan XL, LKB, Bromma, Sweden). Known amounts of purified GSTs were run in parallel with the experimental samples and served as standards for the calculation of the isoenzyme levels of GST α , μ , π and θ in the cytosolic fractions. The detection limit of the western blot assay is approximately 40 ng/mg protein. Total GSH was quantified by high-performance liquid chromatography after reaction with monobromobimane, as described before.²⁸⁾ In this assay, oxidized GSH present is reduced by adding sodium borohydride to the reaction mixture.

Statistics The Wilcoxon signed rank test was used to assess the statistical significance of differences of the various biochemical parameters investigated between Barrett's and adjacent squamous epithelium. Spearman rank correlation analyses was used to correlate the degree of dysplasia with GST enzyme activity and isoenzyme levels in Barrett's epithelium.

RESULTS

Table I shows the GST activity, GST isoenzyme levels and GSH contents of both Barrett's and adjacent normal squamous epithelium. As compared to adjacent normal

Table I. Glutathione S-Transferases and Glutathione in Barrett's and Squamous Epithelium of Patients with Barrett's Esophagus

	Squamous epithelium (n=98)	Barrett's epithelium (n=98)
GST activity (nmol/min-mg protein)	826±23 (293–1480)	539±19* (166–1598)
GST α (ng/mg protein)	274±40 (0–2091)	1987±125* (0–6150)
GST μ (ng/mg protein)	1476±144 (0–4918)	1115±122* (0–7367)
GST π (ng/mg protein)	7034±171 (914–12940)	4931±161* (1050–10510)
GST θ (ng/mg protein)	442±59 (0–4985)	590±30* (0–2271)
GSH (nmol/mg protein)	72±3 (17–141)	27±1* (7–116)

GST activity, GST isoenzymes and GSH were determined as described in "Materials and Methods." Values are given as mean±SEM. Ranges are indicated in parenthesis. *Significantly different when compared to normal esophageal epithelium ($P < 0.0001$).

squamous epithelium, the GST enzyme activity towards CDNB is significantly lower ($P < 0.0001$) in Barrett's epithelium. This decrease was found in 87 patients, whereas in the remaining 11 patients the GST activity was similar in both tissues. No significant correlation was found between degree of dysplasia and GST activity in Barrett's epithelium.

Class α GSTs are expressed at low levels in normal epithelium (274 ± 40 ng/mg protein). In Barrett's epithelium GST α levels are significantly higher as compared with squamous epithelium ($P < 0.0001$). GST α was undetectable in squamous epithelium of 44 patients and in Barrett's epithelium of 8 patients. Seven patients had no detectable GST α levels at either site. GST μ (GSTM1-1) was undetectable in both tissues of 52 patients (53%). Barrett's epithelium contained lower levels of GST μ as compared to adjacent normal squamous epithelium ($P < 0.0001$). Esophageal GSTs consist mainly of GST π (GSTP1-1; 7034 ± 171 ng/mg protein). This isoenzyme was present at high levels in all samples. In Barrett's epithelium mean GST π level was significantly lower ($P < 0.0001$) and lower levels were seen in 89 patients, whereas in the other 9 patients the GST π levels of Barrett's and squamous epithelium were similar. GST θ levels in Barrett's epithelium were significantly higher as compared with squamous epithelium. Twenty-four patients (24%) did not contain any detectable GST θ (GSTT1-1) in both tissues. No significant correlation was found between any of the GST isoenzyme levels and the degree of dysplasia in Barrett's epithelium.

GSH content of Barrett's epithelium is significantly lower as compared to normal squamous epithelium ($P < 0.0001$). These lower levels were seen in 95 patients. A poor inverse correlation between the degree of dysplasia and the GSH content of Barrett's epithelium was found ($R_s = -0.23$; 95% CI = $0.06-0.39$; $P = 0.01$). However, no significant correlation was seen if the degree of dysplasia was compared with the difference in GSH content between Barrett's and normal squamous epithelium ($R_s = -0.06$; 95% CI = $-0.25-0.13$; $P = 0.53$).

DISCUSSION

Patients with Barrett's esophagus have a 30- to 125-fold increased risk of developing esophageal adenocarcinoma compared with the general population.⁵⁻⁹ GSTs are involved in the protection against chemical carcinogenesis.¹⁶ Four main subclasses of GSTs have been demonstrated in humans: α , μ , π and θ .¹⁶⁻¹⁸ Approximately 40-50% of Caucasians lack GSTM1-1 due to a gene deletion.²⁹ Our observation that approximately half of the patients (53%) did not contain any detectable GSTM1-1 protein, and most probably are of the GSTM1-1 null genotype, agrees well with this. Previous studies have shown

that GSTM1 deletion is more common among patients with colorectal cancer,^{19, 30} squamous cell carcinoma of the lung^{21, 31} and other lung cancers,³²⁻³⁴ although results are conflicting in others.^{35, 36} Recently, a null polymorphism in the θ class of GSTs was discovered, resulting in the absence of a functional *GSTT1* gene.¹⁷ GSTT1 null genotype has been correlated with increased risk of colorectal cancer³⁷ and the age of onset of colon cancer,³⁰ although others deny such a relation.³⁸ We were unable to detect GSTT1-1 protein in 24 (24%) patients with Barrett's epithelium, and these patients most probably are of the GSTT1-1 null genotype, which matches well with literature data.³⁹ The absence of GSTM1 or T1 isoforms in Barrett's tissue however, did not correlate with the degree of dysplasia in this tissue.

We found considerable differences in GSTs between Barrett's and adjacent squamous epithelium. Barrett's epithelium showed a significant reduction in GST enzyme activity by 35%, and GST μ , GST π and GSH levels by 24%, 30%, and 63%, respectively. GST α and θ levels, on the other hand, were increased in Barrett's epithelium by 625% and 33%, respectively. However, these isoforms, in a quantitative sense, represent only a small part of total GSTs. These results match well with a pilot study by us, in which 10 patients with Barrett's esophagus were involved.¹³ Here we studied 98 patients, of which 52% were GST μ negative, 46% showed a decreased level, and only 2% showed a slightly increased GST μ level in Barrett's compared with squamous epithelium.

Barrett's epithelium is often considered as gastric epithelium moving up into the esophagus, whereas most Barrett's metaplasias are of the intestinal type⁴; in our study 95 of 98 patients had Barrett's tissue of the intestinal type. Therefore it should be expected that the GST profiles of Barrett's epithelium might resemble those in duodenal mucosa. A comparison of the GST profile of Barrett's epithelium with earlier data on gastric and duodenal GSTs^{13, 40} however, did not support this hypothesis. GST activity and GST μ levels of Barrett's epithelium correlate well with duodenal values. GST α and π values in Barrett's epithelium mainly resemble those found in gastric cardia and antrum, whereas GSH levels found in Barrett's epithelium are intermediate between duodenal and gastric levels.^{13, 40} Differences in GST enzyme activity and isoform levels in Barrett's epithelium as compared with those in normal esophageal epithelium may be partly explained by the difference between squamous and glandular epithelium; however, as shown above, considerable differences exist between Barrett's and normal gastric or duodenal values (all glandular epithelium) while large differences also exist between normal oral/oropharyngeal⁴¹ and esophageal (all squamous epithelium) tissue.

Although involvement of GSTs in the activation of some compounds such as haloalkanes has been reported,⁴²

GSH and GSTs are correlated with protection against cellular or cytogenetic damage, and low contents of GSH or GSTs in Barrett's mucosa may be a factor of relevance for the increased tumor risk in this tissue. GSH levels in Barrett's tissue tended to decrease slightly as dysplasia progressed. GST activity and isoenzyme levels in Barrett's tissue on the other hand, did not correlate with the degree of dysplasia, which makes the GSTs inapplicable as a tumor marker in individuals with Barrett's epithelium. Higher levels of GSH and GST, however, may be very important in the protection of tissues against carcinogenesis.^{13, 15, 16} Therefore modulation of GSH or GST isoenzyme or enzyme activity levels by supplementation with glutathione, precursors of glutathione, or inducers of GSTs may be of value to prevent malignant transformation of Barrett's epithelium.

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