Enhancement of Glutathione S-Transferase Activity of the Esophagus by Phenols, Lactones, and Benzyl Isothiocyanate

Velta L. Sparnins, Ji Chuan, and Lee W. Wattenberg

ABSTRACT

The effects of feeding p-methoxyphenol, benzyl isothiocyanate, coumarin, a-angelicalactone, 2-tert-butyl-4-hydroxyanisole, and 3-tert-butyl-4-hydroxyanisole on the glutathione S-transferase activity and sulfhydryl levels of esophagus and small bowel mucosa of ICR/Ha mice have been investigated. p-Methoxyphenol, benzyl isothiocyanate, coumarin, and 2-tert-butyl-4-hydroxyanisole increased glutathione S-transferase activity of the esophagus by 68 to 135%, a-angelicalactone was less effective, and 3-tert-butyl-4-hydroxyanisole had only a small enhancing capacity. All six compounds increased the sulfhydryl levels of the esophagus. The ranking order and magnitude of the enhancing effects of the six compounds on glutathione S-transferase activity are similar for esophagus and forestomach (previously published) but differ from that of the small bowel mucosa. Since esophageal cancer is an important cause of cancer deaths in many parts of the world, information as to factors which can enhance protective systems of this organ may be of value.

INTRODUCTION

GSH3 S-transferase has been studied extensively as a major detoxification system that catalyzes the reaction of a wide variety of electrophiles to GSH (2, 8). Since the reactive ultimate carcinogenic forms of chemical carcinogens are electrophiles, GSH S-transferase takes on considerable importance as a mechanism for carcinogen detoxification. Enhancement of the activity of this system potentially could increase the capacity of the organism to withstand the neoplastic effects of chemical carcinogens (1, 16). Experiments have been carried out aimed at determining if there is a relationship between increased GSH S-transferase activity in a target organ of chemical carcinogenesis and response to the carcinogen. For this purpose, the forestomach of the mouse was used. Members of several classes of inhibitors of BP-induced neoplasia of the mouse forestomach were studied for their effects on the GSH S-transferase activity in that structure. Five of the compounds increased the GSH S-transferase activity of the forestomach 78 to 182%. The 5 compounds were p-methoxyphenol, 2-BHA, coumarin, a-angelicalactone, and benzyl isothiocyanate. All 5 inhibited BP-induced neoplasia of the forestomach. These data indicate that enhancement of the GSH S-transferase activity by about 75% or greater is associated with a reduced carcinogenic response of the forestomach to BP (14). The data also suggest that the capacity to enhance GSH S-transferase activity might be used as a method of identifying compounds or natural products likely to inhibit BP or other carcinogens detoxified in a similar manner (18).

The forestomach of the mouse is lined by a stratified squamous epithelium without other structures, such as glands, being present. The epithelium is similar to that of the esophagus. However, their milieu is not the same. In addition, epithelia with similar morphology do not necessarily have the same biochemical composition. The esophagus is a particularly interesting organ in that in several areas of the world, including parts of China, esophageal cancer is a leading cause of cancer deaths (3, 10, 13, 15, 19). In the present investigation, the effects on esophageal GSH S-transferase activity and acid-soluble sulfhydryl levels of compounds previously shown to cause increases in both of these entities in the forestomach have been studied. Parallel studies of inhibition of carcinogenesis have not as yet been undertaken.

MATERIALS AND METHODS

Animal Experiments. Female ICR/Ha mice from the Madison, Wis., facility (Harlan Sprague-Dawley, Indianapolis, Ind.) were used in all experiments. Mice were randomized by weight at 7 weeks of age. At that time, they were divided into groups of 5 animals each and placed on experimental diets. Two basic diets were used. One was a semi-purified diet consisting of 27% vitamin-free casein, 59% starch, 10% corn oil, 4% salt mix (U.S.P. XIV), and a complete mixture of vitamins (Teklad Inc., Madison, Wis.). The other was powdered Purina rat chow (Ralston Purina Company, St. Louis, Mo.). To these diets were added the test compounds. The diets were fed for 9 days, at which time the experiment was terminated. Mice were killed at 9 a.m. The esophagus and proximal half of the small bowel were removed. The small bowel was opened, washed, and the mucosa was scraped off gently. In some experiments, the test compounds dissolved in cottonseed oil were given by p.o. intubation once a day for 3 days, the last administration being 24 hr prior to killing the mice.

Preparation of Homogenates and Cytosol. All steps were done at 0-4°. The tissues to be studied were homogenized in 0.1 M phosphate buffer (pH 7.5). The homogenate was centrifuged at 100,000 x g for 1 hr. The supernatant was used for the assay of cytosolic GSH S-transferase activity.

Determinations of GSH S-Transferase Activity and Acid-soluble Sulfhydryl Levels. The activity of cytosol GSH S-transferase was determined spectrophotometrically at 30° with 1-chloro-2,4-dinitrobenzene as substrate, according to the procedure of Habig et al. (7). The reaction mixture (1 ml) contained 100 µmol phosphate buffer (pH 6.5), 5 µmol GSH, and 1 µmol 1-chloro-2,4-dinitrobenzene. The reaction was started by addition of cytosol. There are multiple GSH S-transferases that have been identified in the cytosol of tissues. However, the esophagus has not been investigated in this regard. The known multiple forms of GSH S-transferase have low substrate specificities which...
overlap. 1-Chloro-2,4-dinitrobenzene is a substrate for almost all of the
known GSH S-transferases (2, 8). The major activity is in the cytosol.
These considerations led to the choice of the procedure used in the
present study.

Tissue levels of acid-soluble sulfhydryl groups were determined by
the method of Elman (6). Homogenates were prepared with an
equal volume of 4% 5-sulfosalicylic acid, and the precipitate was
removed by centrifugation. Free sulfhydryl groups were assayed by
the addition of 0.9 ml of 0.1 mM 3,3′-dithiobis(2-nitrobenzoic acid) in 0.1
M phosphate buffer (pH 8.0) to 0.1 ml of the supernatant and then by
the determination of the absorbance at 412 nm. Statistical significance
was determined by use of Student's t test.

Chemicals. Benzyl isothiocyanate was obtained from Eastman Or-
ganic Chemicals (Rochester, N. Y.); coumarin, α-angelica lactone, p-
methoxyphenol, and 1-chloro-2,4-dinitrobenzene were from Sigma
Chemical Co. (Milwaukee, Wis.); and GSH, 5,5′-dithiobis(2-nitroben-
zoic acid), and 5-sulfosalicylic acid were from Sigma Chemical Co. (St.
Louis, Mo.). BHA was also purchased from Sigma. 3-BHA was prepared
from the commercially obtained 2(3)-BHA by recrystallization, and the
2-BHA isomer was synthesized as previously described (9).

RESULTS
Of the compounds included in the present study, p-methox-
yphenol, benzyl isothiocyanate, coumarin, and 2-BHA were the
most potent in enhancing GSH S-transferase activity of the
esophagus. α-Angelica lactone had a lesser enhancing effect
and 3-BHA was the least active. All of the compounds also
increased the acid-soluble sulfhydryl levels in the esophagus
(Table 1). In Experiment 3, a semipurified diet was used rather
than the unpurified diet used in other experiments. The en-
hancing effect of p-methoxyphenol on GSH S-transferase and
acid-soluble sulfhydryl levels of the esophagus was greater
with the semipurified diet than with the unpurified one. In
other experiments, it was found that 3 daily administrations of test
compounds by p.o. intubation resulted in weaker enhancing
effects than when these chemicals were given in the diet,
according to the standard procedure used in the present stud-
ies.

A comparison of the effects of the test compounds on the

Table 1
Effects of several chemicals on GSH S-transferase activity and acid-soluble sulfhydryl levels in the esophagus and small bowel mucosa of the mouse

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Test compound</th>
<th>Esophagus</th>
<th>Small bowel mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSH S-transferase activity</td>
<td>Acid-soluble sulfhydryl level</td>
<td>GSH S-transferase activity</td>
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<tr>
<td></td>
<td>µmol/min/mg protein</td>
<td>µmol/g tissue</td>
<td>Relative value</td>
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<tr>
<td>1</td>
<td>None</td>
<td>0.61 ± 0.02</td>
<td>1.70 ± 0.05</td>
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<td></td>
<td>p-Methoxyphenol</td>
<td>1.38 ± 0.10</td>
<td>2.26 ± 0.18</td>
</tr>
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<td>2</td>
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<td>0.61 ± 0.02</td>
<td>2.20 ± 0.05</td>
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<td>p-Methoxyphenol</td>
<td>1.48 ± 0.04</td>
<td>2.43 ± 0.09</td>
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<td>3</td>
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<td>0.76 ± 0.03</td>
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<td></td>
<td>3-BHA</td>
<td>2.19 ± 0.01</td>
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<td>GSH S-transferase activity</td>
<td>3.92 ± 0.02</td>
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<td>0.68 ± 0.03</td>
<td>1.52 ± 0.11</td>
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<td>Benzyl isothiocyanate</td>
<td>1.78 ± 0.02</td>
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<td>2-BHA</td>
<td>1.20 ± 0.02</td>
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<td>0.64 ± 0.02</td>
<td>1.46 ± 0.09</td>
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<td>1.79 ± 0.07</td>
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<td>0.70 ± 0.03</td>
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<td>3-BHA</td>
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<td>0.49 ± 0.03</td>
<td>1.30 ± 0.05</td>
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<td></td>
<td>p-Methoxyphenol</td>
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<td>1.59 ± 0.12</td>
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<tr>
<td></td>
<td>3-BHA</td>
<td>0.64 ± 0.04</td>
<td>1.24 ± 0.09</td>
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</table>

GSH S-transferase activity was assayed according to the method of Habig et al. (7), using 1-chloro-2,4-dinitrobenzene as the substrate.

a Acid-soluble sulfhydryl level was assayed according to the method of Elman (6).

b For 9 days prior to sacrifice, mice were fed an unrefined diet (powdered Purina rat chow) with the indicated additions. Test compounds were added in the amount of 0.03 mmol/g of diet.

c Mean ± S.D. p < 0.05.

d Mean ± S.D. p < 0.05.

e Mean ± S.D. p < 0.05.

f For 9 days prior to sacrifice, mice were fed a semipurified diet with the indicated additions. Test compounds were added in the amount of 0.03 mmol/g of diet.

g Cottonseed oil (0.2 ml) with 0.03 mmol of the test compound was administered to mice daily for 3 days prior to sacrifice. Controls received cottonseed oil only.

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GSH S-transferase activity and acid-soluble sulfhydryl levels of the esophagus and mucosa of the small bowel shows that differences exist. These differences are particularly evident with the phenols. 3-BHA is potent for small bowel mucosa and weak for esophagus. \( \beta \)-Methoxyphenol is weak for small bowel mucosa but potent for esophagus.

**DISCUSSION**

In the present study, it has been shown that consumption of diets containing benzyl isothiocyanate, \( \beta \)-methoxyphenol, 2-BHA, \( \alpha \)-angelicalactone, and coumarin all result in substantial enhancement of GSH S-transferase activity and acid-soluble sulfhydryl levels in the esophagus of female ICR/Ha mice. The same compounds all enhance GSH S-transferase activity in the mouse forestomach under comparable experimental conditions (14). The ranking order and magnitude of enhancement of GSH S-transferase activity by the compounds included in the present study was similar for esophagus and forestomach but not identical. The GSH S-transferase activity ratios of test to control mice for the forestomach after the feeding the two compounds were: 2-BHA, 2.82; benzyl isothiocyanate, 2.46; \( \beta \)-methoxyphenol, 2.45; \( \alpha \)-angelicalactone, 1.95; coumarin, 1.78; and 3-BHA, 1.14. For the esophagus, the corresponding ratios were: \( \beta \)-methoxyphenol, 2.35; benzyl isothiocyanate, 2.34; coumarin, 1.89; 2-BHA, 1.68; \( \alpha \)-angelicalactone, 1.41; and 3-BHA, 1.21. The most notable difference was with 2-BHA, which had a greater enhancing activity on the GSH S-transferase activity of the forestomach than on the esophagus. The effects of the compounds on the mucosa of the small bowel differ considerably from that of both the esophagus and forestomach.

Compounds that enhance GSH S-transferase activity in the forestomach by approximately 75% or greater inhibit BP-induced neoplasia of that structure. \( \beta \)-Methoxyphenol, benzyl isothiocyanate, coumarin, and 2-BHA enhance the GSH S-transferase activity of the esophagus by 68 to 135%. If the same levels of induction of GSH S-transferase activity in the esophagus as in the forestomach result in protection against carcinogens, these 4 compounds would be expected to have the capacity to diminish the impact of neoplastic agents on the esophagus. Carcinogen inhibition studies in the esophagus have not been carried out as yet. Of considerable importance in this regard is the question as to whether inhibition of nitrosamine-induced neoplasia of the esophagus can be obtained, since the esophagus is a prime target of many nitrosamines (4). Epidemiological data suggest that this class of carcinogens may be involved in causation of human esophageal neoplasia, since a number of nitrosamines have been detected in the diet of high-risk areas of esophageal cancer (5, 11, 12). Two of the compounds investigated in the present study, \( \alpha \)-benzyl isothiocyanate and coumarin, are naturally occurring constituents of food (17). It is possible that dietary constituents altering levels of GSH S-transferase activity might diminish the effects of chemical carcinogens on the esophagus.

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**REFERENCES**


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