Glycoprotein Metabolism in Normal Proximal and Distal Rat Colon and Changes Associated with 1,2-Dimethylhydrazine-induced Colonic Neoplasia

Hugh J. Freeman, Yongwhan Kim, and Young S. Kim

Gastrointestinal Research Laboratory, Veteran's Administration Hospital and University of California, San Francisco, California 94121

ABSTRACT

Glycoproteins are major components of secreted mucins as well as membranes of gastrointestinal mucosal cells. Both qualitative and quantitative differences in mucosal cell glycoproteins from different regions of the colon have been suggested by histochemical studies. Glycoprotein alterations have also been reported in human colon cancer. Since regional differences in glycoprotein synthesis are apparent in rat small intestine, the mucosal sugar content as well as the activities of several glycosyltransferases and glycosidases from both proximal and distal colonic sites in the rat were examined. Differences in total hexose content as well as the activities of several glycosyltransferases and some glycosidases were found, indicating that glycoprotein content and metabolism are different between these two sites. These regional differences were then considered in relation to colon carcinogenesis induced by 1,2-dimethylhydrazine. Significant alterations in hexose content and two galactosyltransferase activities were observed in uninvolved colonic mucosa following administration of this carcinogen. Furthermore, significant reductions in sugar content, three of four glycosyltransferase activities and two hexosaminidases were observed in colonic tumors. These data indicate that significant alterations in glycoproteins occur in association with chemical carcinogenesis in both colonic tumors as well as in uninvolved rat colonic mucosa. Furthermore, these studies emphasize the importance of considering the regional differences in quantity and quality of glycoproteins in normal colonic mucosa when studying pathological states.

INTRODUCTION

Colonic cancer is the major cause of mortality and morbidity from gastrointestinal cancer in the United States (33). Information related to this disorder in the past has been derived to a great extent from clinical observation. Recently, however, the development of colonic neoplasia in experimental animals with chemical carcinogens has provided an additional means of investigating some of the biological characteristics of these tumors (36).

The remarkable similarity of these rodent tumors induced by chemical carcinogens such as 1,2-dimethylhydrazine to human neoplasia is well established (5, 28, 34). With this carcinogen, the vast majority of colonic tumors occur distally, paralleling the distribution of human tumors (32, 34). Furthermore, similarities in the histopathology of these rat tumors, at least in distal colon, are present compared to human neoplasms in distal colon (34). In proximal colon, these apparent similarities do not appear to be as clearly defined. At this site, the tumors are more variable in histopathological type, many being of the mucinous variety of adenocarcinoma (34). The latter variety is only very rarely seen in rat distal colon (34). The specific reasons for this predilection of colonic neoplasia to distal colon as well as the differences in morphological type between these 2 sites are not clear. However, both the structure and function of these 2 regions of the colon differ significantly in both humans (2, 6-8, 21) and experimental animals (10, 24, 29-31). These inherent regional differences between proximal and distal colon may be critical in determining some of the apparent biological differences in tumors originating from these 2 different sites.

Differences in glycoprotein metabolism (11, 12, 18) appear to be present in human colonic cancer tissue compared to tissue obtained from normal-appearing adjacent colonic mucosa. In human colonic tumors, carbohydrate content was found to be reduced in association with reduced activities of some glycosyltransferases involved in the synthesis of the carbohydrate portion of mucosal glycoproteins (11, 12). This study was undertaken to further elucidate these biochemical changes with rats with colonic neoplasia induced by 1,2-dimethylhydrazine. In view of the known structural and functional differences occurring in proximal and distal colon, however, the carbohydrate content and enzyme activities from these 2 sites were first examined. Subsequently, these results were compared to those obtained from normal-appearing colonic mucosa as well as from tumor-bearing tissues from rats treated with 1,2-dimethylhydrazine.

MATERIALS AND METHODS

Animals and Carcinogenesis. Male Wistar rats (Simonson Laboratories, Inc., Gilroy, Calif.) weighing 100 to 120 g, maintained on laboratory chow pellets (Ralston Purina Co., St. Louis, Mo.), were given injections weekly of 1,2-dimethylhydrazine dihydrochloride (Aldrich Chemical Co., Inc., Milwaukee, Wis.) at a dosage of 25 mg/kg of body weight from age 6 to 24 weeks. This was prepared as a 0.5% solution of 1 mM EDTA (Mallinckrodt Inc., St. Louis, Mo.) ad-
justed to pH 6.5 with sodium bicarbonate. Control animals received an equivalent amount of EDTA of identical pH. Preliminary studies in our laboratory in a separate group of rats had established this to be effective for tumor induction in most rats as reported elsewhere by others (32, 34). Animals were sacrificed between 8:00 and 10:00 a.m. at 52 weeks of age by rapid etherization and decapitation. The large intestine was rapidly removed in a cold room maintained at 4°C, gently rinsed with cold 0.9% sodium chloride solution and divided into proximal (caecum and ascending colon) and distal (descending colon and rectum) portions. Proximal and distal portions of the rat colon may be distinguished grossly by the presence of oblique mucosal folds in the former and longitudinal folds in the latter (34). Histologically, tumors in the distal portion of the colon are mainly polyoid neoplasms or adenocarcinomas as classified by Ward (34). Tumors in the proximal colon occur less frequently in this model, appear to be variable in pathological type, and include the mucinous variety (34). For this reason, our attention was directed to the distal colon where tumors are more numerous and of a similar histopathological type. For carbohydrate analysis, mucosal scrapings of grossly normal proximal and distal colon from controls and carcinogen-treated rats as well as distal colonic tumors from the carcinogen group were homogenized (10 times v/w) in 50 mM cacodylate buffer, pH 6.5, with the Polytron homogenizer. All homogenates were stored separately at -20°C until needed. Prior to aliquoting for chemical analysis and enzyme assays as well as the protein determinations, homogenates were always sonically disrupted.

Chemical Analysis of Carbohydrates. Total mucosal hexose was determined by the method of Winzler (37) with glucose as the standard while total mucosal hexosamine was determined by the modification of Boas (1) of the method of Elson and Morgan (4) with glucosamine-hydrochloride (Mann Research Laboratories, New York, N. Y.) as the standard. Subsequently, mucosal homogenates were dialyzed for 48 hr with distilled water, and nondialyzed tissues and distal colonic tumor tissues from carcinogen-treated animals were homogenized (10 times v/w) in 50 mM cacodylate buffer, pH 6.5, with the Polytron homogenizer. All homogenates were stored separately at -20°C until needed. Prior to aliquoting for chemical analysis and enzyme assays as well as the protein determinations, homogenates were always sonically disrupted.

Glycosyltransferase Assays. The activities of 4 glycosyltransferases were measured in the mucosal homogenates essentially as described previously (11-15, 17, i.e., galactosyltransferases I and II and sialyltransferases I and II. The acceptors used for the glycosyltransferases were: for galactosyltransferase I, asialo-agalactosyl-olive submaxillary mucin; for galactosyltransferase II, asialo-agalactosyl-\(\alpha\)-acid glycoprotein; for sialyltransferase I, asialo-\(\alpha\)-acid glycoprotein. Conditions for the enzyme assays were modified slightly to obtain linearity of the reaction with respect to time and amount of enzyme.

Glycosidase Assays. The activities of 5 glycosidases (9, 27) were measured at their pH optimum as follows: \(\alpha\)-galactosidase, pH 3.6; \(\beta\)-galactosidase, pH 4.0; \(\beta\)-N-acetylgalactosaminidase, pH 4.7; \(\beta\)-N-acetylgalactosaminidase, pH 5.7; and \(\alpha\)-mannosidase, pH 4.6, with 4-methylumbelliferyl derivatives of 5 sugars (Koch-Light Laboratories, Colnbrook, England). The reaction mixture contained 20 nmol of 4-methylumbelliferyl glycose, 20 \(\mu\)g of enzyme protein, and 100 \(\mu\)l of 0.1 M acetic acid buffer with 0.2% Triton X-100 in a final volume of 200 \(\mu\)l. The mixture was incubated at 37°C for 10 or 20 min depending on the substrate, and the reaction was then terminated by addition of 2.0 ml of ice-cold 20 mM glycine-5 mM EDTA buffer, pH 10.4. The free 4-methylumbelliferyl was measured at an excitation wavelength of 365 nm and an analyzer wavelength of 460 nm with a Perkin-Elmer fluorimeter. In addition, derivatives of 3-nitrophenylglycosides were also used as substrate for \(\beta\)-galactosidase, \(\beta\)-N-acetylgalactosaminidase, and \(\beta\)-N-acetylgalactosaminidase, and activities were examined as previously described (25) with some modifications. The assay mixture contained 80 to 100 \(\mu\)g of enzymatic protein (instead of 0.5 to 1.0 mg), 200 nmol of a 3-nitrophenylglucoside (instead of 20 nmol), and 50 umol of sodium citrate buffer, pH 4.2, in a final volume of 1.0 ml. The reaction was terminated after 30 min (instead of 1 hr) by adding 1.0 ml of cold 0.4 M glycine-NaOH buffer, pH 10.5. The released free 3-nitrophenol was measured at 400 nm with a Bausch & Lomb spectrophotometer. All enzyme assays were performed under conditions in which the reaction was linear with respect to enzyme concentration and time.

Data Analysis. Results were expressed on the basis of protein as determined by the method of Lowry et al. (22) with crystalline bovine serum albumin as standard and analyzed statistically with the Student t test.

RESULTS

Carbohydrate Composition of Colonic Mucosa and Tumors. As shown in Table 1, sugar content was greater in distal colon compared to proximal colon, although statistical significance could only be achieved for total and nondialyzable mucosal hexose (\(p < 0.02\)). In colonic mucosa from rats given injections of 1,2-dimethylhydrazine but free of detectable tumors, sugar content was similar to control normal rats except for total hexose content. Total proximal and distal mucosal hexoses were statistically increased compared to normal control proximal (\(p < 0.05\)) and distal mucosa (\(p < 0.05\)). In addition, in carcinogen-injected rats, distal colonic hexose content was greater than the content determined for proximal colon (\(p < 0.05\)). In tumors removed from the distal colon, tumor sugar content (total hexose, hexosamine, and sialic acid) was reduced compared to the sugar content of nontumorous distal colonic mucosa from both normal controls (\(p < 0.005, p < 0.005, and p < 0.01\), respectively) and carcinogen-injected animals (\(p < 0.005, p < 0.001, and p < 0.025\), respectively). Similarly, nondialyzable hexose and hexosamine fractions as well as...
bound sialic acid were lower in tumors compared to normal
distal mucosa (p < 0.05, p < 0.005, and p < 0.05, re-
spectively) and to carcinogen-injected animals (p < 0.05,
p < 0.02, and p < 0.01, respectively).

**Glycosyltransferase Activities.** Table 2 shows the levels
of 4 glycosyltransferase activities from colonic mucosa and
tumors of normal controls and carcinogen-injected rats. Ex-
cept for galactosyltransferase I, all other glycosyltrans-
ferases (galactosyltransferase II, sialyltransferase I, and
sialyltransferase II) had reduced activities in distal colon
(p < 0.025, p < 0.005, and p < 0.05, respectively). In com-
paring the mucosa of carcinogen-injected rats to normal
controls, activities were statistically greater in proximal
colon for both galactosyltransferase (p < 0.001 for both)
but remained similar for both sialyltransferases. The activi-
ties of 3 glycosyltransferases (galactosyltransferase I, sialy-
transferase I, and sialyltransferase II) were significantly
lower in tumor tissue from distal colon compared to the ac-
tivities in distal colonic mucosa of normal control rats (p <
0.02, p < 0.001, and p < 0.025, respectively). Galactosyl-
transferase II activity, however, was increased compared to
normal distal colonic mucosa (p < 0.005).

**Glycosidase Activities.** Table 3 shows the levels of 5
glycosidases with 4-methylumbelliferone sugar derivatives
while Table 4 shows the levels of 3 glycosidases with p-
nitrophenyl sugar derivatives. Similar results for these 2
different methods of glycosidase activity determination
were obtained in this study. Both hexosaminidase activities
(β-N-acetylgalcosaminidase and β-N-acetylgalactosami-
dase) were increased in distal colonic mucosa compared to
proximal colonic mucosa of normal control rats (p < 0.005
for both with either method) while the activities of 2 galac-
tosidases and mannosidase were no different. While no
significant differences were detected in tumor-free proximal
colonic mucosa of carcinogen-injected rats, tumors had
reduced activities of β-N-acetylgalcosaminidase and β-N-
acetylgalactosaminidase compared to normal control colo-
nic mucosa with either 4-methylumbelliferone (p < 0.001).

### Table 1

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Normal rats (n = 8)</th>
<th>Tumor-free proximal mucosa</th>
<th>Tumor-free distal mucosa</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal mucosa</td>
<td>Distal mucosa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexose</td>
<td>1.07 ± 0.16a</td>
<td>1.70 ± 0.14b</td>
<td>1.56 ± 0.21b</td>
<td>2.25 ± 0.18e</td>
</tr>
<tr>
<td></td>
<td>(0.49 ± 0.09)f</td>
<td>(0.83 ± 0.06)</td>
<td>(0.52 ± 0.09)</td>
<td>(0.81 ± 0.09)</td>
</tr>
<tr>
<td>Hexosamine</td>
<td>0.94 ± 0.05</td>
<td>1.17 ± 0.17</td>
<td>1.12 ± 0.09</td>
<td>1.21 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>(0.54 ± 0.05)</td>
<td>(0.56 ± 0.04)</td>
<td>(0.50 ± 0.05)</td>
<td>(0.52 ± 0.05)</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>0.18 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(0.13 ± 0.03)</td>
<td>(0.14 ± 0.02)</td>
<td>(0.12 ± 0.01)</td>
<td>(0.15 ± 0.01)</td>
</tr>
</tbody>
</table>

a Mean ± S.E. (μmol/mg protein).
b p < 0.05, t test versus proximal colon from normal control rats.
c p < 0.05, t test versus distal colon from normal control rats.
d p < 0.05, t test versus 1,2-dimethylhydrazine proximal colon.
e p < 0.05, t test versus 1,2-dimethylhydrazine distal colon.
f Numbers in parentheses, nondialyzable sugar content for hexose and hexosamine and bound fraction for sialic
acid (35).

### Table 2

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal rats (n = 6)</th>
<th>Rats given 1,2-dimethylhydrazine injections (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal mucosa</td>
<td>Distal mucosa</td>
</tr>
<tr>
<td>Galactosyltransferase I (Gal-GalNAc–)</td>
<td>12.9 ± 1.5b</td>
<td>15.7 ± 2.0</td>
</tr>
<tr>
<td>Galactosyltransferase II (Gal-GlcNAc–)</td>
<td>20.7 ± 3.7</td>
<td>9.5 ± 1.7c</td>
</tr>
<tr>
<td>Sialyltransferase I (NANA-GalNAc–)</td>
<td>5.5 ± 1.0</td>
<td>2.3 ± 1.1c</td>
</tr>
<tr>
<td>Sialyltransferase II (NANA-Gal–)</td>
<td>32.3 ± 8.9</td>
<td>13.8 ± 1.2c</td>
</tr>
</tbody>
</table>

a Proximal colonic mucosa free of grossly visible tumors and colonic tumor mucosa from
distal colon.
b Mean ± S.E.
c p < 0.05, t test versus proximal colon from normal control rats.
d p < 0.05, t test versus distal colon from normal control rats.
e The sugar that is transferred and the terminal sugar of the acceptor are shown.
Table 3
Glycosidases of colonic mucosa from normal rats and rats given 1,2-dimethylhydrazine injections
Values represent enzyme activities (nmol/mg protein per min \times 10^{-2}). 4-Methylumbelliferone derivatives of 5 sugars were used for determination of glycosidase activities.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal rats (n = 6)</th>
<th>Rats given 1,2-dimethylhydrazine injections (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal mucosa</td>
<td>Distal mucosa</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>5.1 ± 1.8^b</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>8.3 ± 0.9</td>
<td>10.0 ± 0.9</td>
</tr>
<tr>
<td>β-N-Acetylglucosaminidase</td>
<td>16.2 ± 2.1</td>
<td>28.3 ± 2.2^c</td>
</tr>
<tr>
<td>β-N-Acetylgalactosaminidase</td>
<td>6.8 ± 1.0</td>
<td>12.7 ± 0.9^c</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>2.6 ± 0.5</td>
<td>3.3 ± 0.2</td>
</tr>
</tbody>
</table>

^a Proximal colonic mucosa free of grossly visible tumors and colonic tumor mucosa from distal colon.
^b Mean ± S.E.
^c p < 0.05, t test versus proximal colon from normal control rats.
^d p < 0.05, t test versus distal colon from normal controls rats.

Table 4
Glycosidases of colonic mucosa from normal rats and rats given 1,2-dimethylhydrazine injections
Values represent enzyme activities (nmol/mg protein per min). p-Nitrophenyl derivatives of 3 sugars were used for determination of glycosidase activities.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal rats (n = 6)</th>
<th>Rats given 1,2-dimethylhydrazine injections (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal mucosa</td>
<td>Distal mucosa</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>3.8 ± 0.5^b</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>β-N-Acetyl glucosaminidase</td>
<td>10.4 ± 1.7</td>
<td>28.6 ± 3.9^c</td>
</tr>
<tr>
<td>β-N-Acetylgalactosaminidase</td>
<td>3.0 ± 0.4</td>
<td>6.6 ± 0.5^c</td>
</tr>
</tbody>
</table>

^a Proximal colonic mucosa free of grossly visible tumors and colonic tumor mucosa from distal colon.
^b Mean ± S.E.
^c p < 0.05, t test versus proximal colon from normal control rats.
^d p < 0.05, t test versus distal colon from normal control rats.

DISCUSSION

Regional differences between normal proximal and distal rat colon were present in relation to mucosal carbohydrate content and enzymes involved in glycoprotein metabolism. Sugar content was greater distally, although statistical significance could only be achieved in this study for total and nondialyzable hexose. Furthermore, significant regional differences were demonstrated for most glycosyltransferases and some degradative glycosidase enzyme activities. Specific correlations, however, between the results of these sugar determinations and the levels of enzyme activities are difficult for two reasons: (a) the total carbohydrate measured in this study reflects both free and membrane-bound sugars associated with the glycoprotein and glycolipid fractions of the colonic mucosa; and (b) the glycoprotein acceptors and synthetic substrates used may not accurately reflect the natural acceptors and substrates occurring in mucosal cells of the rat colon. Nevertheless, the observed regional differences in carbohydrate content and enzyme activities are important in considering glycoprotein metabolism in animals receiving chemical carcinogens such as 1,2-dimethylhydrazine. These differences in carbohydrate content between proximal and distal colon are consistent with earlier chemical and histochemical studies of the colonic mucosa from both humans (6-8, 21) and experimental animals (10, 24, 29-31). Regional variations in the levels of enzymes involved in glycoprotein synthesis have been observed in the small intestine of the rat (16). However, one earlier report (26) failed to document any regional differences in glycosidase activities with p-nitrophenyl derivatives in rat colon. In this study with both p-nitrophenyl and umbelliferone derivatives, consistent elevations in β-N-acetylglucosaminidase and β-N-acetylgalactosaminidase were seen while 3 other glycosidase activities remained unchanged.

Histochemical studies (3, 6, 8, 19, 23) have suggested that considerable differences in carbohydrate content of mucinous glycoproteins may occur between normal colonic...
mucosa, uninvolved adjacent “traditional” mucosa, and colon tumors. In this study, when apparently tumor-free mucosa from proximal colon and tumor-bearing distal colon were examined, hexose (total and nondialyzable) content of tumor-free tissue was increased compared to the same region in normal rats while results for hexosamine (total and nondialyzable) and sialic acid (total and bound) were similar. In comparing the results of enzyme activities from tissue at similar proximal sites, a marked increase in both galactosyltransferase activities were present in carcino-ogen-treated rats. Sialyltransferase as well as glycosidase activities, however, remained unchanged. Thus carcinogen treatment seemed to have a differential effect on the type of sugar and enzymes involved in glycoprotein metabolism. These results indicate that during chemical carcinogenesis considerable alteration in the metabolism of glycoproteins occurs in colonic mucosa.

When colonic tumors were compared to normal and carcinogen-treated uninvolved mucosa, all 3 sugars (both total and nondialyzable or bound forms) were significantly reduced. The observed reduction in sugar content was associated with reductions in 3 of 4 glycosidase activities. These results are consistent with the previously reported findings in human colonic cancer (11, 12) and in experimental rat tumors induced by 1,2-dimethylhydrazine (20). The activity of galactosyltransferase II was not reduced in tumors compared to distal colonic mucosa from normal rats. The activity of this enzyme was not measured in the carcino-pentreated distal colonic mucosa due to limited amount of tissue. It is likely, however, that this enzyme was unchanged or decreased in distal colon tumors compared to tumor-free, carcinogen-treated distal colon since the levels of both galactosyltransferases were increased 2- to 3-fold with carcinogen treatment.

Compared to distal colonic mucosa from normal rats, reduced activities of hexosaminidase occurred in tumors while other glycosidases (examined in this study) remained unchanged. In previous studies on glycosidases in human colonic cancer (11, 12), the levels of activities remained unchanged except for β-N-acetylgalactosaminidase (12). If we had compared the results of these 2 glycosidase activities in this study to proximal colonic mucosa from both normal and carcinogen-treated rats, no significant differences would have been observed. These results, therefore, further emphasize the importance of comparing equivalent colonic sites because of regional differences.

In summary, the results of this study appear to indicate that regional differences in glycoprotein content and metabolism occur in normal rat colon. These differences require consideration in colonic disease processes including neoplasia. Whether the observed alterations are primary events or reflect changes secondary to neoplastic transformation requires further investigation.

REFERENCES

H. J. Freeman et. al.


Glycoprotein Metabolism in Normal Proximal and Distal Rat Colon and Changes Associated with 1,2-Dimethylhydrazine-induced Colonic Neoplasia

Hugh J. Freeman, Yongwhan Kim and Young S. Kim


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/38/10/3385

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.