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Measurement of sialyl-transferase activity in isolated colonic mucosal cells of the rat

I. O. OLUBUYIDE, A. P. CORFIELD, J. B. RAINEY* AND J. R. CLAMP
Departments of Medicine and Surgery, Bristol Royal Infirmary, Bristol, U.K.*

Summary

An assay for sialyl-transferase activity in isolated rat colonic mucosal cells has been developed. Total and specific activity with asialo-bovine sub-mandibular gland glycoprotein and endogenous substrates was approximately two-fold higher in the proximal, relative to the distal, colon. These activities were similar when asialo-α-acid glycoprotein was used as substrate. Endogenous activity was approximately 10-fold lower than with exogenous substrates in both proximal and distal colonic segments. Analysis of total and specific sialyl-transferase activity up to 7 weeks after jejuno–ileal bypass (JIB) and sham operation showed a marked increase at the first week, decreasing towards normal by week 7. Similar differences between proximal and distal colon, and with the type of substrate, were found with both groups of operated animals. Histochemical analysis showed small elevations after 1 week for sialo and sulphomucins after the JIB operation only.

Résumé

Un essai pour l'activité sialyl-transférase dans les cellules mucosaux du colon d'un rat isolé a été développé. L'activité totale et spécifique d'une glande glycoprotéine submandibulaire asialo-bovine et les substrates endogéniques étaient presque deux fois plus grands dans le proximal en relation au colon distal. Ces activités étaient similaires quand le glycoprotéine asialo-acide a été utilisé comme substrate. L'activité endogénique était en plus bas presque dix fois qu'avec les substrates exogéniques dans tous les deux segments du colon (proximal et distal). L'analyse totale et spécifique de l'activité sialyl-transférase jusqu'au sept semaines après en dérivation (JIB) jéjuno–iléal et une opération a montré une augmentation dans la première semaine diminuant à normal dans la septième semaine. Les différences similaires ont été trouvé dans le colon proximal et distal avec le type de substrates dans les animaux opérés. L'analyse histochemical a montré un petit élévation après une semaine pour sialo et sulphomucines dans l'opération JIB seulement.

Correspondence: Dr I. O. Olubuyide, Department of Medicine, University College Hospital, Ibadan, Nigeria.

Introduction

The goblet cells of the colonic mucosa are responsible for the formation of the mucus glycoprotein secreted into the colonic lumen. The mucus glycoproteins contain sialic acid and have been well documented histochemically [1,2].

Sialyl-transferase activity has been reported in rat and human colon, and changes have been detected which are associated with malignant disease [3–6]. The histochemical changes observed during malignancy have also been described, and particularly reflect the change in sialic acid moieties in the diseased tissue [7,8].

Histochemical analyses of colonic mucosal hyperplasia in ulcerative colitis [9–11] appear similar to those found in the colon after the jejuno–ileal bypass (JIB) operation [12,13]. As no biochemical analysis of diseased or operated tissue for sialyl-transferase activity has been reported it was decided to develop such an assay for isolated colonic mucosal cells, and assess its value in the first few weeks after JIB and sham operation as a monitor of mucosal change.

Materials and methods

Eighteen male Sprague–Dawley rats (Olac Ltd, Bicester, U.K.) weighing 300.2 ± 4.1 g (mean ± s.e.m.) were randomly allocated to receive sham bypass (jejunal transection, ileotomy and resuture), and (85%) JIB, the equivalent of the operation used for the treatment of morbid obesity in man. The animals received oxoid SGI diet (H. G. Styles and Sons, Bewdley, U.K.) and water *ad libitum* throughout the experiment. Quarters were lit in alternate 12 h-cycles. Four non-operated animals were killed at the beginning of the experiment and two each at 1, 3 and 7 weeks, while 1-week post-operation and subsequently at 2-week intervals, two operated animals (one sham bypass and one rat with JIB) were killed by ether anaesthesia. Laparatomy was performed and the entire colon removed. The colon was opened longitudinally and divided into proximal and distal segments. The segments were mopped and weighed. For histochemical studies, 1-cm morphometric segments were cut from the middle of the proximal and distal colon and stored in 10% formalin. For enzyme studies, the mucosal cells were isolated from the colon by a method described previously [14]. Each colonic segment was washed thoroughly with phosphate-buffered saline (PBS) at 20°C, and incubated for two periods of 15 min with 1 mM dithiothreitol in PBS at 20°C, and the supernatant discarded. The cleaned segment was incubated twice in batches of PBS containing 1.5 mM EDTA for 90 min at 37°C. All the incubating solutions contained an antibiotic mixture [14]. The cells were liberated from the colon by EDTA and remaining tissue removed, the cells were then sedimented by centrifigation. The pellet was homogenized in 0.15 M NaCl at 5°C using a Polytron at setting 6 for 10 sec, and centrifuged at 100,000 *g* for 60 min at the same temperature. The membrane and supernatant fractions were separated using a Pasteur pipette and the membrane pellet dispersed in 0.154 M NaCl.

To determine the sialyl-transferase activity in the proximal and distal colon, 150 μg of the membrane pellet derived from each colonic segment was incubated with a mixture containing 10 μl (0.03 μmol, 0.045 μCi) CMP-(4-^{14}C) sialic acid (New England Nuclear, Dreieich, F.R.G.), 10 μl asialo substrate; either asialo-α-acid glycoprotein (20 mg/ml) (Scottish National Blood Transfusion Service, Edinburgh, U.K.) or bovine sub-mandibular gland asialomucus glycoprotein (BSM) (10 mg/ml) as prepared by Tettamanti and Pigman [15] and Corfield *et al.* [16]; 5 μmol Na cacodylate, 10 μmol $MnCl_2$, 50 μg bovine serum albumin and 0.5 mg Triton X-100 at pH 6.5 in a total volume of 50 μl. After 120 min, the reaction was stopped by adding 500 μl of ice-cold 5% phosphotungstic acid in 15% trichloracetic acid. The mixture was centrifuged at 12,000 *g* for 5 min and the pellet was washed twice with 1 ml of 95% ethanol. It was dissolved overnight in 0.2 ml of 0.2 M NaOH at room temperature, and the radioactivity measured by liquid scintillation counting in 10 ml of dioxan-based scintillation fluid. The enzyme activity was determined by the amount of radioactivity in the precipitated proteins and expressed as nmol [^{14}C]sialic acid transferred/mg protein/h. All assays and blanks were carried out in duplicate. These consisted of incubating boiled enzyme with either asialo substrates and CMP-(4-^{14}C) sialic acid before adding the stop solution or adding active enzyme to the substrate mixture directly before the stop solution. Endogenous activity was measured by omitting the asialo substrates and making up the volume with distilled water. The values were subtracted from results obtained using exogenously added substrates. Protein was assayed by the method of Lowry *et al.* [17]. All membrane pellets were measured in duplicate at three dilutions and values from the initial linear portion of the curve used. Bovine serum albumin was used as standard.

The morphometric segments were routinely embedded in paraffin and two serial 5-μm histological sections were cut from at least three levels in the specimen blocks and were stained with high iron diamine–alcian blue for sialomucins and sulphomucins [18] and periodic acid Schiff's (PAS) reagent for neutral mucins. The number of goblet cells containing sialomucins, sulphomucins or PAS reactivity was estimated for each coded slide using 10 perfectly sectioned crypts per slide.

Results

Experiments with non-operated rats established the conditions for the sialyl-transferase assay in

proximal and distal colonic mucosal cells. The activity in proximal and distal colonic cells was linearly related to the time of incubation up to 3 h with both of the exogenously added substrates (asialo-α-acid glycoprotein and asialo-BSM), and endogenous activity was linear up to 4 h. After these times deviation from linearity was observed. Linearity with protein concentration was found in all incubations up to 200 μg. The pH optimum of the activity with all three substrates was 6.5 in proximal and distal colon using two buffer systems.

The routine assay based on these findings used up to 150 μg protein at pH 6.5 in cacodylate buffer for 2 h at 37°C.

The data obtained for normal rats are shown in Table 1 and are incorporated into the results in Fig. 1. A difference in total sialyl-transferase activity and the specific activity was observed between proximal and distal colon with asialo-BSM and endogenous activity. No difference was found for asialo-α-acid glycoprotein. The number of goblet cells containing sialomucins was 60% higher in the proximal colon relative to the distal, but no differences were found for proximal and distal sulphomucin and PAS reactivity (Fig. 2).

The short-term effect of surgery on histochemical staining and sialyl-transferase activity in proximal and distal colon is shown in Figs 1 and 2. Elevation of sialyl-transferase activity was evident after only 1 week with all substrates in both the proximal and distal colon (Fig. 1). The increase in total activity had dropped towards initial levels by the third week except for the distal colon with asialo-BSM and endogenous activities (Fig. 1b, c). Differences in total sialyl-transferase activity between sham and JIB operated rats were evident for the proximal and distal colon with asialo-α-acid glycoprotein (Fig. 1a), and for proximal colon with asialo-BSM (Fig. 1b) after 1 week. The variation in the distal colon was less marked and not limited to the first week for asialo-BSM and endogenous substrates (Fig. 1b, c).

Histochemical analysis showed elevation of sialomucin and PAS reactive staining in proximal and distal JIB operated rats (Fig. 2a, c). Sulphomucin staining showed elevation in the distal colon after the JIB operation (Fig. 2b) and a reduction in the amount of sialomucin staining found in the distal colon of sham-operated rats (Fig. 1a). No other changes were evident and all values except sham-operated sialomucin staining tended to return to initial values by the seventh week.

The levels of total endogenous activity in normal rats were low (Table 1) but the elevations observed were the largest (Fig. 1c). The activity paralleled the changes found with the asialo-BSM substrate.

The wet weight ratio of proximal to distal colon showed similar levels to the value found for non-operated rats in both sham and JIB operated rats with exceptions at 3 and 7 weeks (Table 2). The ratio of membrane pellet

Table 1. Sialyl-transferase activity in non-operated rats

Substrate	Sialyl-transferase activity (nmoles/h/mg protein)		
	Proximal colon	Distal colon	*n*
Asialo-α-acid glycoprotein	0.056 ± 0.011	0.055 ± 0.009	
	(0.73 ± 0.05)	(0.85 ± 0.09)	10
Asialo-bovine submandibular gland glycoprotein	0.067 ± 0.015	0.023 ± 0.006	
	(0.78 ± 0.10)	(0.33 ± 0.04)	10
Endogenous	0.005 ± 0.003	0.002 ± 0.002	
	(0.064 ± 0.015)	(0.034 ± 0.032)	10

The specific activity of sialyl-transferase with each of the three substrates is shown. The figures in parentheses are for total sialyl-transferase (nmol/h/total protein) for the corresponding substrates.

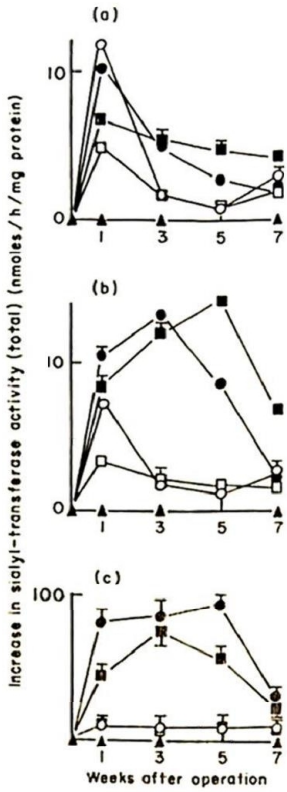

Fig. 1. Influence of operation on total sialyl-transferase activity in proximal and distal colonic cells. Total sialyl-transferase activity for non-operated (▲), sham (○, ●) and JIB (□, ■) animals in proximal (○, □) and distal (●, ■) colon. The increase is calculated from the non-operated animals at day one in proximal and distal colon for (a) asialo-α-acid glycoprotein, (b) asialo-bovine submandibular gland glycoprotein, and (c) endogenous substrate. Absolute values are given in Table 1. Standard deviation is shown by bars for each sample where necessary.

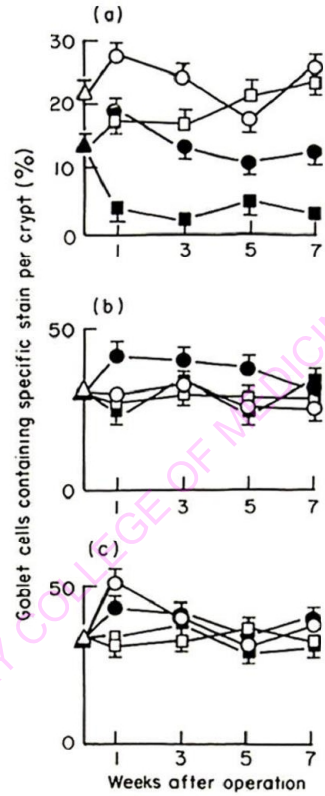

Fig. 2. Histochemical analysis of proximal and distal colonic goblet cell staining after operation. The percentage of goblet cells staining for (a) sialomucin, (b) sulphomucin, and (c) PAS reactivity per crypt, in non-operated (△, ▲), sham (○, ●) and JIB (□, ■), for proximal (△, ○, □) and distal (▲, ●, ■) colon is shown. Standard deviation is indicated by bars.

protein from proximal and distal colon dropped in the first 3 weeks and normalized after 5 weeks, while the total membrane protein for proximal and distal colon appeared to return to initial values in the sham experiment but remained elevated in the JIB operation (Table 2).

Discussion

Sialyl-transferase activity in the rat colon has been detected using an assay system with two exogenous asialo-substrates and endogenous activity. The exogenous substrates were chosen to reflect different sialyl-transferase activities known to exist with respect to mucus (asialo-BSM) and non-mucus (asialo-α-acid) glycoprotein substrates [19]. The endogenous activity detectable is low but reflects the nature of sialyl-transferase to colonic mucosal cell glyco-conjugates present in the isolated membranes.

The normal range of total and specific activity found in rat colon varied with the colonic segment and the nature of the substrate assayed (Table 1) in agreement with Freeman *et al.* [4] and endogenous activity showed a similar pattern to the mucus glycoprotein substrate.

Table 2. Proximal and distal colon wet weight and membrane protein before and after surgery

	Weeks after operation				
	0	1	3	5	7
Ratio of wet weight (proximal:distal)					
Non-operated	1.85 ± 0.3	1.81 ± 0.6	1.9 ± 0.2	—	1.80 ± 0.6
Sham operated	—	2.5	1.1	2.0	2.8
JIB operated	—	2.0	2.2	2.2	1.6
Ratio of membrane protein (proximal:distal)					
Non-operated	0.84 ± 0.2	0.86 ± 0.2	0.91 ± 0.3	—	0.82 ± 0.15
Sham operated	—	0.26	0.38	0.82	0.73
JIB operated	—	0.39	0.45	0.80	0.87
Total membrane protein (proximal + distal)					
Non-operated	28.5 ± 5.1	30.5 ± 1.6	30.6 ± 4.5	—	29.3 ± 3.1
Sham operated	—	58.1	19.2	36.2	30.9
JIB operated	—	81.5	34.0	40.8	55.8

Wet weight was measured for the proximal and distal colon after having been removed, washed and blotted. Membrane protein was that protein sedimented at 100,000 *g* after homogenization of mucosal cells isolated from the colonic segments. There were two rats in each group of non-operated animals except for the week 0 group which contained four.

The assay is sensitive to changes in activity and is ideally suited to monitor changes occurring during carcinogenesis, disease or injury.

The suitability of the assay system is illustrated in monitoring the early changes in sialyltransferase activity after JIB and sham operation. In contrast to the decreased levels of enzyme activity found in 1,2 dimethylhydrazine-induced tumours in rats, a rapid increase is observed after 1 week in all operated rats with all substrates (Fig. 1). Levels returned towards initial values by 7 weeks and the pattern of elevation and decrease was characteristic for each substrate, with endogenous similar to asialo-BSM activity and these both distinct from asialo-α-acid glycoprotein patterns. The response to surgery is similar in both JIB and sham-operated animals. Histochemical results show similar increases relative to sialyl-transferase activity and these are only observed in the JIB operation. Sham operation leads only to a decrease in sialomucin staining in the distal colon. It is likely that the increase in sialyl-transferase activity is due to surgical shock [20] as well as adaptation to the nature of the operation [21] as sham-operated activity was also elevated and showed a similar response pattern to the JIB operation. Elevation of specific sialyl-transferase activity indicates *de novo* synthesis of sialyl-transferase in response to surgery. This is of further interest as the site of enyzme synthesis was removed from the site of surgery.

Studies on glycoprotein biosynthesis in the gastrointestinal tract have revealed regional variation in normal [22], pathological [23] and cancer [3,4,22] tissue. This report demonstrates the suitability of a sialyl-transferase assay to follow such changes in colonic mucosal glycoprotein biosynthesis.

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