Biochimica et Biophysica Acta, 673 (1981) 435—442
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BBA 29544

ELEVATED SIALYLTRANSFERASE ACTIVITY IN THE INTESTINAL LYMPH OF COLCHICINE-TREATED RATS

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(Received August 1st, 1980)

Key words: Sialyltransferase; Colchicine treatment; (Rat lymph)

Summary

There is a marked increase of sialyltransferase activity (EC 2.4.99.1) in serum and a profound change in the endogenous acceptor property of sialyltransferase in the intestine of colchicine treated rats (Fraser, Ratnam, Collins and Mookerjea, (1980) J. Biol. Chem. 255, 6617—6625). To ascertain the contribution of intestine as a source of this elevated serum enzyme, sialyltransferase and other enzyme activities were measured in intestinal lymph before and after colchicine treatment. There was a 4-fold increase of the enzyme activity in lymph 3 h after treatment. The lymph flow rate, protein concentration and composition as measured by polyacrylamide gel electrophoresis were not affected. The kinetic properties of lymph sialyltransferase (protein and time dependence, pH optima and K_m values for the substrate CMP-sialic acid) were essentially unchanged after treatment and were similar to the serum sialyltransferase. Alkaline phosphatase and lactic dehydrogenase activities were elevated in the lymph whereas maltase and lactase activities remained unchanged. Although intestinal lymph sialyltransferase was increased by colchicine, enterectomy did not prevent the rise of serum sialyltransferase suggesting that the intestine is not a major source of the serum enzyme.

Introduction

Colchicine, injected intraperitoneally into rats, caused a 7 to 10-fold increase of sialyltransferase (EC 2.4.99.1) activity in serum within 17 h [1]. Intestinal

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homogenates from colchicine treated rats showed a small increase of the enzyme activity, but a much larger increase of endogenous sialyltransferase activity was observed when the homogenates were incubated for several hours [2]. The present work was designed to ascertain if there was any causal relationship between the changes in the enzyme activity in serum and in intestine following colchicine treatment. Lymph originating from the small intestine was collected at different time intervals before and after colchicine treatment by cannulating the main lymphatic duct draining the small intestine and the sialyltransferase and other enzyme activities were measured. Sialyltransferase activity in lymph was increased soon after colchicine treatment which suggested that the increase of enzyme activity in lymph might have resulted as a consequence of changes in the intestine. This change in lymph may, in turn, lead to observed elevation of sialyltransferase in serum. However, there is also evidence contrary to this possibility. Properties of the serum and intestinal sialyltransferase activities in colchicine treated rats have been shown to be different [2] and, therefore, it is unlikely that one could be a source of the other. Also, the colchicine induced increase of sialyltransferase in serum was evident even when the rats were enterectomized thus reducing the possibility of intestine acting as a source of the serum enzyme.

Materials and Methods

CMP-sialic acid (spec. act. 1.68 mCi/mmol) was purchased from New England Nuclear Corporation (Dorval, Quebec). Purified human α-1 acid glycoprotein was a gift from the American Red Cross National Fractionation Centre, Washington, D.C., and fetuin was purchased from Sigma (St. Louis, MO). Fetuin was more than 90% pure and α_1-acid glycoprotein gave a single band by polyacrylamide gel electrophoresis [9]. Exogenous acceptor for the assay of sialyltransferase was prepared by the removal of sialic acid from α-1 acid glycoprotein or fetuin by a chemical method [3]. Colchicine and other reagents used were obtained from Sigma Chemical Co. (St. Louis, MO).

The main lymphatic vessel draining the small intestine was cannulated in anaesthetised adult Sprague-Dawley rats weighing 200—250 g according to the method of Warshaw [4] as modified by Turner and Barrowman [5]. The duodenum was cannulated close to the pylorus with an infant feeding tube (Blue line infant feeding tube, Portex, U.K.), and 0.9% NaCl was infused constantly at the rate of 2.5 ml/h. An intravenous line was established in the tail vein with PE 10 tubing (Clay-Adams, U.S.A.) and saline was infused at 0.5 ml/h. After operation, animals were kept in restraining cages in a warm room with free access to drinking water but no food [6].

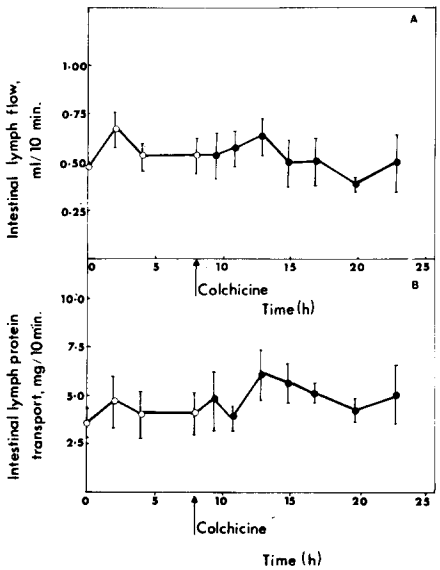
Experiments were performed on the first post-operative day. Intestinal lymph was collected in graduated citrated tubes for 10 min at zero time and at 2 h intervals thereafter up to 8 h. Colchicine prepared freshly in 0.9% saline was then injected (0.25 mg/100 g body wt.) intravenously through the tail vein cannula. Intestinal lymph was collected similarly for 10-min periods at definite intervals up to 12—20 h. The duodenal infusion of saline served to offset the loss of fluid from the lymphatic fistula. For studies of enterectomized animals a laparotomy was performed under ether anaesthesia and the small intestine

was removed from the duodenum to the ileo-cecal valve. The cut ends of the duodenum and ileo-cecal region were tied off. Minimal bleeding occurred as the vessels were ligated. Sialyltransferase activity was assayed in lymph (25—50 μl, 250—450 μg protein) or serum (10 μl, 700 μg protein) by the method described previously using desialylated fetuin or desialylated α_1-acid glycoprotein as exogenous acceptors [1,2]. These two acceptors gave identical results and they were used in different sets and in duplicate experiments. In other experiments two other desialylated glycopeptides prepared from human IgC were used to show the dramatic elevation of sialyltransferase in serum after colchicine treatment [2]. The validity of the methods used for assay has also been examined by high voltage paper electrophoresis to obtain the radioactive products. Alkaline phosphatase and lactic dehydrogenase activities were assayed according to the method described in the Worthington Enzyme Manual [7]. Disaccharidases were assayed by the method of Dahlqvist [8]. Analytical polyacrylamide gel electrophoresis of lymph samples was run as described [9]. Protein was measured by the method of Lowry et al. [10] using bovine serum albumin as standard.

Results

Experiments were conducted in groups of 3 to 5 rats. Intestinal lymph collected at definite intervals up to 8 h prior to colchicine treatment gave a flow rate of 0.55 ± 0.05 ml/10 min, and the lymph protein transport at the rate of 4.2 ± 0.46 mg/10 min (Fig. 1). Following the injection of colchicine 8 h after zero time, the lymph collected from the same animals at various time intervals showed no significant changes either in the flow rate or in protein concentration. After the drug treatment, the average flow rate of lymph was 0.64 ± 0.12 ml/10 min. Since protein concentration did not alter, there was no change in the total amount of protein carried in the lymph which averaged 4.96 ± 0.37 mg/10 min. The lymph samples obtained before and after colchicine treatment showed identical protein profiles on polyacrylamide gels (Fig. 2).

The assay for sialyltransferase in the lymph samples showed a sharp increase in the enzyme activity during the first few hours after colchicine treatment (Fig. 3). The activity was elevated 2-fold 1.5 h after the treatment, and 4-fold by 3 h. The enzyme activity remained elevated about 4.5-fold until the termination of the experiment 15 h after colchicine treatment. Sialyltransferase activity is linear with protein concentration and with time of incubation in the lymph collected before and 12 h after the drug treatment (results not shown). The effect of pH on the enzyme assay is shown in Fig. 4. The maximum enzyme activity was observed using Mes (2-(*N*-morpholino)ethane sulfonic acid) buffer of pH 6.8. The rate of enzyme activity was studied as a function of CMP-sialic acid concentration and data was analyzed by the method of Lineweaver and Burk [11]. There was little change in the apparent K_m values of the enzyme for the substrate, 0.86 mM and 1.32 mM before and after colchicine treatments respectively. The V of the lymph enzyme was however increased about 4.5-fold (from 0.18—0.83 nmol). Triton X-100 when included in the assay in final concentrations ranging from 0.25—1.0% showed a slight inhibitory effect on the enzyme activity. The enzyme assays performed without the



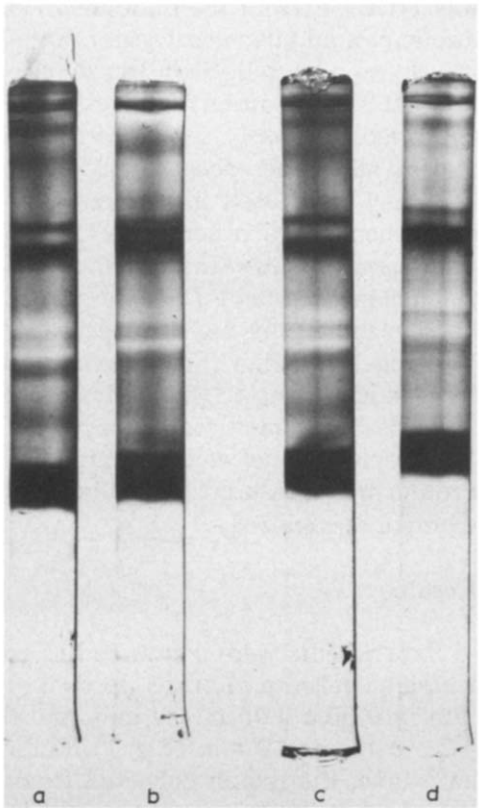


Fig. 1. Effect of colchicine treatment on intestinal lymph flow (A) and protein transport (B) in rat. Colchicine was injected intravenously, 0.25 mg/100 g body weight 8 h after lymphatic cannulation (shown by an arrow). Lymph was collected before (○) and after colchicine treatment (●) at different intervals and the volume and protein concentration was determined as described in the text. Values on the graph represent the mean of three to five animals and the vertical bars indicate S.E.

Fig. 2. Polyacrylamide gel electrophoresis of rat lymph. Lymph samples collected before and 12 h after colchicine treatment as described in the text were electrophoresed on polyacrylamide gels by the method of Davis [9]. (a) control lymph; (b) lymph after colchicine treatment. Both these samples were dialysed overnight at 4°C in Tris (5 mM)/glycine (38 mM) buffer pH 8.3 before they were electrophoresed; (c) and (d) control and colchicine treated as above, but the lymph samples were not dialysed. For each gel about 70 μg of protein was applied.

exogenous acceptor protein indicated an absence of endogenous acceptor activity in the lymph collected before and after colchicine treatments (results not shown). To find out if the control lymph and the lymph samples collected after colchicine treatment contained any specific inhibitory or stimulatory factor for the sialyltransferase, mixing experiments were carried out. The results of these experiments (Table I) does not indicate presence of any such factors. The properties of lymph sialyltransferase as presented here are, therefore, very similar to the serum enzyme [1].

The disaccharidases, i.e., maltase and sucrase activities assayed in the lymph collected before and at 12 h after colchicine treatment showed no changes due to colchicine treatment (Table II). However, the activities of lactic dehydro-

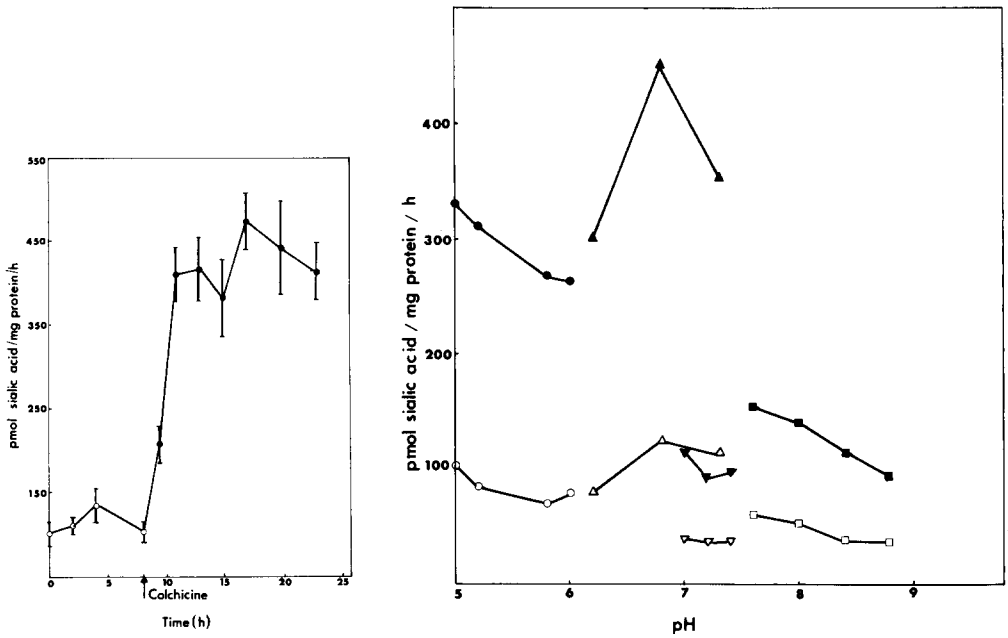


Fig. 3. Effect of colchicine treatment on lymph sialyltransferase activity in rat. Lymph was collected before (○) and after colchicine treatment (●) as described for Fig. 1. Sialyltransferase activity was assayed as described in the text. Values on the graph represented the mean of three to five animals and the vertical bars indicate S.E.

Fig. 4. Effect of varying pH on the assay of lymph sialyltransferase activity. Lymph collected before (open symbols) and 12 h after colchicine treatment (closed symbols) was assayed for sialyltransferase activity using buffers at different pH as indicated below. Citrate-PO_4 ○, ●; Mes △, ▲; Cacodylate ▽, ▼; Tris-HCl □, ■. Concentration of buffers used was 125 mM.

TABLE I

RESULT OF THE MIXING EXPERIMENT USING THE LYMPH COLLECTED BEFORE AND 12 h AFTER COLCHICINE TREATMENT

Lymph samples were preincubated for 30 min at 37°C after mixing in different proportions. Then the assay ingredients were added and the enzyme activity was measured as described in the text.

Experiment	Sialyltransferase activity (cpm/h)		
	Actual values	Sum of mixed assay	Difference
10 μl, control lymph	64.4		
10 μl, colchicine lymph	146.6		
10 μl, control + 10 μl, colchicine lymph	225.9	211.0	+6.6%
30 μl, control lymph	200.5		
30 μl, colchicine lymph	599.9		
30 μl, control + 30 μl, colchicine lymph	708.4	800.4	—11.6%
10 μl, control + 30 μl, colchicine lymph	698.8	664.3	+4.9%
30 μl, control + 10 μl, colchicine lymph	369.5	347.1	+6.1%

TABLE II

EFFECT OF COLCHICINE TREATMENT ON DISACCHARIDASES, ALKALINE PHOSPHATASE AND LACTIC DEHYDROGENASE ACTIVITIES IN RAT LYMPH

Lymph collected before and 12 h after colchicine treatment was assayed for the various enzyme activities as described in the text. Values represent the mean of 3—5 rats.

	Maltase [a] (U/mg protein)	Sucrase [a] (U/mg protein)	Lactic dehydrogenase [b] (U/g protein)	Alkaline phosphatase [c] (U/g protein)
Control	5.03 ± 0.46	1.46 ± 0.06	0.83 ± 0.09	2.47 ± 0.29
After colchicine treatment	4.90 ± 0.10	1.47 ± 0.11	2.07 ± 0.17 [d]	4.67 ± 0.88

[a] One unit is that activity hydrolysing 1 μmol disaccharide per min under the defined conditions at 37°C.

[b] One unit of activity is that which causes an initial rate of oxidation of 1 μmol of $NADH_2$ per min under the conditions specified at 25°C.

[c] One unit is that activity liberating 1 μmol *p*-nitrophenol per min under the defined conditions at 25°C.

[d] Significant statistical difference from the control value $P < 0.01$.

TABLE III

EFFECT OF ENTERECTOMY ON THE COLCHICINE INDUCED INCREASE OF SIALYLTRANSFERASE ACTIVITY IN SERUM

Enterectomies were performed as described in the Materials and Methods section. Colchicine (0.5 mg/100 g wt.) or NaCl 0.9% was injected via the tail vein the morning following surgery. Serum was taken 4 h later. The serum sialyltransferase value 17 h after colchicine injection (0.5 mg/100 g wt.) intraperitoneally is shown for comparison.

Treatment	Sialyltransferase activity in serum (pmol sialic acid per mg protein per h)
Control	127 ± 28
Colchicine, 4 h	221 ± 25
Colchicine, 17 h	835 ± 183
Enterectomy, 18 h	270 ± 53
Enterectomy, 18 h + colchicine, 4 h	566 ± 131
Sham surgery, 18 h + colchicine, 4 h	584 ± 100

genase and alkaline phosphatase were found elevated about 2-fold 12 h after colchicine.

Results on the effect of colchicine on serum sialyltransferase activity of the various control and enterectomized animals are shown in Table III. Enterectomized animals showed an increase of sialyltransferase activity in serum which was further increased after colchicine treatment. Animals with sham surgery plus colchicine also showed a similar increase of the serum enzyme activity. These results suggest that the small intestine is not the primary source of the increased serum sialyltransferase observed in response to colchicine treatment.

Discussion

In rat, intestinal lymph flows primarily via a single-lymphatic vessel [4], and cannulation of this vessel ensures sampling of most of the lymph draining from

the small intestine. Although colchicine is known to inhibit secretion of proteins by liver [12,13], there was no significant change in the protein concentration of the lymph, nor was a change in the rate of lymph flow noticed (Fig. 1).

It has been previously shown that the increase of sialyltransferase in the serum after colchicine treatment continued almost linearly up to 24 h [1], whereas the results obtained for lymph showed that the increase reached a plateau within 2 to 3 h (Fig. 2). It is possible that the capacity to saturate the level of enzyme proteins in the lymph compartment is quite low compared to serum. Various studies on the source of lymph and lymph proteins have suggested that they are mainly derived from plasma filtration [14]. However, our data on the early increase of the enzyme activity in lymph after colchicine treatment (2-fold in 1.5 h) compared to a 50% increase in serum in 2—3 h [1] might suggest intestinal lymph as a potential contribution to the serum enzyme. It is imperative that intestinal origin of the lymph enzyme activity be established to advance the argument of a temporal relationship between the lymph and serum enzyme levels. However, the following evidence appears to contradict such a possibility. Elevated enzyme activity in lymph and serum required an exogenous acceptor, i.e., DS-fetuin, for activity. In contrast, the newly exposed sialyltransferase in intestinal tissue of the cochicine treated rats is of endogenous type and did not use DS-fetuin of other exogenous acceptors for activity [2]. The intestinal enzyme, therefore, is different from the serum or lymph enzyme. Furthermore, the results presented in this paper (Table III) clearly showed that colchicine induced increase of sialyltransferase activity in serum is not prevented in enterectomized rats, which argues against the possibility of an intestinal source of elevated serum enzyme activity. The elevated levels of alkaline phosphatase and lactic dehydrogenase (Table II) may suggest some cell damage, possibly in the liver, due to colchincine and this may result in the leakage of these cytoplasmic enzymes into the serum and hence to lymph. Moreover, serum alkaline phosphatase and 5'-nucleotidase activities were elevated following colchicine treatment (unpublished results). It has also been noted that a sialyltransferase from bovine colostrum has been purified and is composed of two species with molecular weights of 56 000 and 43 000 [15]. Molecules of this size could be expected to filter fairly readily from blood to lymph [14].

Several reports in recent years of elevated levels of serum glycosyltransferases in disease states, especially in neoplastic diseases, have focussed attention on the significance of these changes in serum as reflection of abnormal cellular function [16—21]. The strikingly elevated level of sialyltransferase observed in serum and lymph of colchicine treated rats has offered an excellent model to study the relationship between membrane related changes and release or shedding of cellular glycosyltransferases. It is further intriguing that among serum glycosyltransferase enzymes only sialyl- and to a smaller extent fucosyltransferase levels have been shown to increase following colchicine treatment. Since sialic acid and fucose are terminal sugars in oligosaccharide chains, this observation may lead to better understanding of the processes of cell renewal and membrane fusion. Possibilities of a relationship between changes in sialyltransferase activity and rapid renewal of intestinal cells are now under investigation [2,22,23]. Sialyltransferase activity in the tissues is membrane-bound and mainly localized in the Golgi membranes. Therefore, release of this membrane-

bound enzyme into the serum and lymph in a soluble form may represent a physiological mechanism in response to colchicine treatment. It is also interesting that colchicine treatment has triggered the release or shedding mechanism of only sialyltransferase (and to a smaller extent fucosyltransferase) and this enzyme is probably situated at a distal morphological site in the membranes. The special localization of sialyltransferase on the membranes may very well suit its function to modulate the intramembranous recognition mechanism and this mechanism is probably affected by disruption of microtubules as a result of colchicine treatment.

Acknowledgement

This research is supported by grants from the Medical Research Council of Canada and the Canadian Heart Foundation.

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