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The role of sphingomyelin synthetase and sphingomyelinase in 1,2-dimethylhydrazine-induced lipid alterations of rat colonic plasma membranes

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Recently, our laboratory, utilizing the 1,2-dimethylhydrazine model of colonic adenocarcinoma, demonstrated alterations in the 'dynamic component' of fluidity in brush-border membranes prepared from distal colonocytes of rats administered this agent for 5, 10 and 15 weeks, i.e., before the development of colon cancer. Furthermore, changes in the sphingomyelin content and sphingomyelin / phosphatidylcholine molar ratio of these membranes appeared, at least partially, to be responsible for these fluidity alterations. In an attempt to elucidate the mechanism(s) involved in these dimethyhydrazine-induced lipid changes, in the present studies the activities of sphingomyelin synthetase and magnesium-dependent neutral sphingomyelinase, enzymes involved in the synthesis and degradation of this phospholipid, respectively, were examined and compared in distal colonic brush-border membranes prepared from rats after 5, 10 or 15 weeks administration of dimethylhydrazine or diluent. The results of these studies demonstrate that alterations in both these enzymatic activities can be detected after administration of dimethylhydrazine and appear to, at least in part, be responsible for the changes in membrane sphingomyelin composition noted previously. These results as well as a discussion of their possible significance serve as the basis for the present report.

Considerable attention has been focused recently on changes in plasma membranes and intracellular membranes that occur during the malignant transformation process [1–8]. Alterations in membrane phospholipid and neutral lipid composition [1–5] and fluidity [5–8] have been demonstrated in various cells undergoing malignant transformation. In this regard, our laboratory, utilizing the 1,2-dimethylhydrazine model of colonic adenocarcinoma, have recently shown that changes in the 'dynamic' component of fluidity, as assessed by steady-state fluorescence polarization techniques using r values of the probes DL-2-(9-anthroyl)stearic acid (2-AS) and DL-12-(9-anthroyl)stearic acid (12-AS), could be detected in distal colonic brush-border membranes before the development of colon cancer [9]. Furthermore, these 'premalignant' changes in fluidity, at least in part, appeared to be due to alterations in the lipid composition of dimethylhydrazine-treated membranes, particularly their sphingomyelin content, sphingomyelin phosphatidylcholine molar ratio and arachidonic acid levels [9]. The mechanism(s)

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responsible for these membrane lipid changes, however, were not clear at that time [9].

In an attempt to elucidate the mechanism(s) involved in the alterations in sphingomyelin content, in the present studies, we examined the activities of sphingomyelin synthetase (phosphatidylcholine: ceramide cholinephosphotransferase) and magnesium-dependent neutral sphingomyelinase, enzymes responsible for the synthesis [10] and degradation [11] of this phospholipid, respectively, in distal colonic brush-border membranes after 5, 10, and 15 weeks of administration of dimethylhydrazine. The results of these studies given below demonstrate that alterations in both the enzymatic activities can be detected in these plasma membranes after administration of dimethylhydrazine and therefore appear to, at least partially, be responsible for the changes in membrane sphingomyelin composition noted previously [9].

Albino male rats of the Sherman strain weighing 75–100 g were given weekly s.c. injections of diluent or 1,2-dimethylhydrazine dihyrochloride (Sigma Chemical Co., St. Louis, MO) at a dose of 20 mg/kg body wt. for 5, 10 and 15 weeks as described [9]. At 5-week intervals, control and dimethylhydrazine-treated animals were fasted for 18 h with water ad libitum before they were killed. For each control or dimethylhydrazine-treated preparation, six animals were killed rapidly by cervical dislocation, their colons excised, the cecum from each animal discarded, and the remaining large intestine divided into two parts: proximal and distal [12]. Epithelial cells were then obtained from the distal segment [13], pooled, and used to isolate brush-border membranes as previously described [14]. The purity of each membrane preparation was assessed by the marker enzyme alkaline phosphatase (*p*-nitrophenylphosphatase) [14]; specific activity ratios [(purified membranes)/(original homogenates)] ranged from 12 to 15 for this enzyme in each preparation and did not significantly differ between control and treated preparations. The corresponding values for NADPH: cytochome *c* reductase, succinate dehydrogenase, and sodium potassium-dependent adenosine triphosphatase, marker enzymes for microsomal, mitochondrial and basolateral membranes, respectively, ranged from 0.40 to 1.30 in each of these preparations [14]. Protein was estimated by the method of Lowry et al. [15] using bovine serum albumin as standard.

Sphingomyelin synthetase specific activity in colonic brush-border membranes was determined by the method of Nelson and Murray [10]. Colonic brush-border membrane neutral sphingomyelinase activity was measured according to a modified procedure of Hostetler and Yazaki [16]. The incubation medium contained 50 mM Tris-HCl (pH 7.2), 2.0 mM [^{14}C]sphingomyelin (65 000 cpm/tube), 2.5 mg/ml Triton-X-100, 40 mM $MgCl_2$ and 40–60 μg membrane protein in a final volume of 200 μl. The reaction mixture was incubated at 37°C for 20 min and terminated by the addition of 3 ml of chloroform/methanol (2 : 1, v/v). Release of ^{14}C-labeled ceramide was then estimated according to the procedure of Yamaguchi and Suzuki [17]. Assay conditions were chosen to assure linear kinetics and and excess of substrate, i.e., maximal velocity, throughout the test period and no more than 5% of the substrate was utilized in reaction period. The reactions were also linear with respect to time and protein concentrations used in these experiments.

In our previous studies [9], after 5-week administration of dimethylhydrazine, no significant differences in the sphingomyelin content or sphingomyelin/phosphatidylcholine molar ratio were noted in distal brush-border membranes prepared from control and dimethylhydrazine-treated membranes. After 10- and 15-week administration of dimethylhydrazine, however, the sphingomylin content and sphingomyelin/phosphatidylcholine molar ratio in distal dimethylhydrazine-treated colonic membranes were both found to be significantly greater than in their control membrane counterparts [9]. As shown in Table I, in the present studies at the 5-week period neither the specific activities of sphingmyelin synthetase nor neutral sphingomyelinase were found to be different in colonic distal control and dimethylhydrazine-treated brush-border membranes. After 10 and 15 weeks of administration of dimethylhydrazine, however, the specific activities of sphingomyelin synthetase and neutral sphingomyelinase were found to be significantly higher and lower, respectively, in colonic dimethylhydrazine-treated membranes than their control

TABLE I

SPHINGOMYELIN SYNTHETASE AND SPHINGOMYELINASE SPECIFIC ACTIVITIES IN BRUSH-BORDER MEMBRANES PREPARED FROM DISTAL COLONOCYTES AFTER 5, 10 AND 15 WEEKS OF ADMINISTRATION OF DIMETHYLHYDRAZINE OR DILUENT TO SHERMAN RATS

Values represent means ± S.E. of six determinations of three separate preparations of each membrane.

Duration of treatment (weeks)	Sphingomyelin synthetase (nmol/mg protein per h)		Sphingomyelinase (nmol/mg protein per h)	
	Control	Dimethylhydrazine	Control	Dimethylhydrazine
5	5.2 ± 0.8	4.3 ± 0.9	261 ± 18	320 ± 42
10	4.6 ± 0.2	8.1 ± 0.2 [a]	221 ± 41	105 ± 15 [a]
15	3.7 ± 0.1	7.6 ± 0.1 [a]	263 ± 8	156 ± 15 [a]

[a] $P < 0.01$ or less compared to control values.

counterparts. Each of these differences in activity, theoretically, should lead to an increase in membrane sphingomyelin content and an increased molar ratio of sphingomyelin/phosphatidylcholine [10,11]. While the mechanism(s) involved in these enzymatic changes are unclear at this time, it seems reasonable to suggest that these alterations in enzymatic activities detected at the 10- and 15-week periods are, at least partially, responsible for the lipid composition changes previously noted at these time periods [9]. These experiments do not, however, preclude other potential mechanism (s) for the increased membrane sphingomyelin content seen in dimethylhydrazine-treated rats. In this regard, prior studies in a rat hepatoma cell line [18] have suggested that increased levels of sphingomyelin in tumor mitochondrial membranes may be due to protein-catalyzed sphingomyelin transfer from the endoplasmic raticulum. Clarification of this issue will, therefore, require additional studies.

The exact role of these membrane lipid alterations in the malignant transformation process in the large intestine also remains unclear at this time. As previously shown by our laboratory [9], these changes in sphingomyelin content, along with alterations in arachidonic acid (20 : 4) levels in dimethylhydrazine-treated membranes, appeared to be, at least partially, responsible for differences in fluidity noted between control and dimethylhydrazine-treated membranes. In this regard, Dawson et al. [19] have also recently shown that changes in sphingomyelin content can influence rat intestinal mucosal phospholipase A_2 activity, thereby, altering arachidonic acid levels. While obviously speculative, these latter observations taken together with the present data suggest that dimethylhydrazine-induced alterations in sphingomyelin synthetase and sphingomyelinase appear to lead to an increase in membrane sphingomyelin content which, in turn, may influence membrane arachidonic acid levels. Further studies along these lines should prove interesting in that arachidonic acid is a precursor of prostaglandins and prostacyclin (PGI_2), substances previously shown to influence cellular proliferation [20–22].

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References

1 Cave, W.T., Jr. and Jorowski, J.J. (1984) J. Natl. Cancer Inst. 73, 185–191
2 Hillyard, L., Rao, G.A. and Abraham, S. (1980) Proc. Soc. Exp. Biol. Med. 164, 376–383
3 Hartz, J.W., Morton, R.E., Waite, M. and Morris, H.P. (1982) Lab. Invest. 46, 73–78
4 Satouchi, K., Mizumo, T., Sanejima, Y. and Saito, K. (1984) Cancer Res. 44, 1460–1464
5 Van Blitterswijk, W.J., De Veer, G., Krol, J.H. and Emmelot, P. (1982) Biochim. Biophys. Acta 688, 495–504
6 Van Blitterswijk, W.J. (1984) in Physiology of Membrane Fluidity (Shinitzky, M., ed.), Vol. 2, Chapter 3, pp. 53–84, CRC Press, Boca Raton, Florida
7 Schroeder, F. (1984) Biochim. Biophys. Acta 776, 299–312
8 Van Hoeven, R.P., Van Blitterswijk, W.J. and Emmelot, P. (1979) Biochim. Biophys. Acta 55, 44–54
9 Brasitus, T.A., Dudeja, P.K. and Dahiya, R. (1986) J. Clin. Invest. 77, 831–840
10 Nelson, D.K. and Murray, D.K. (1982) Proc. Natl. Acad. Sci. USA 79, 6690–6692
11 Schneider, P.B. and Kennedy, E.P. (1967) J. Lipid Res. 8, 202–202
12 Shamsuddin, A.K.M. and Trump, B.F. (1981) J. Natl. Cancer Inst. 66, 375–388

13 Brasitus, T.A. and Keresztes, R.S. (1983) Biochim. Biophys. Acta 728, 11–19
14 Brasitus, T.A. and Keresztes, R.S. (1984) Biochim. Biophys. Act 773, 290–300
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
16 Hostetler, K.Y. and Yazaki, P.J. (1979) J. Lipid Res. 20, 456–463
17 Yamaguchi, S. and Suzuki, K. (1977) J. Biol. Chem. 252, 3805–3813
18 Dyatlovitskaya, E.V., Timofeeva, N.G., Yakimenko, E.F., Barsukov, L.I. Muzya, G.I. and Bergelson, L.D. (1982) Eur. J. Biochem. 123, 311–315
19 Dawson, R.M.C., Hemington, N. and Irvine, R.F. (1985) Biochin. J. 230, 61–68
20 Craven, P.A. and DeRubertis, F.R. (1983) Prostaglandins 26, 583–604
21 Craven, P.A. and DeRubertis, F.R. (1980) Cancer Res. 40, 4589–4598
22 Morisaki, N., Lindsey, J.A., Milo, G.E. and Cornewell, D.G. (1983) Lipids 18, 349–352