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Decreased Levels of 13-Hydroxyoctadecadienoic Acid (13-HODE) Dehydrogenase in Neoplastic Tissue of Human Colon Biopsies[1]

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Abstract

Recent studies have identified a role for the oxidation product of linoleic acid, 13-hydroxyoctadecadienoic acid (13-HODE) in cell proliferation. The enzyme 13-HODE dehydrogenase catalyzes the conversion of 13-HODE to 13-oxooctadecadienoic acid. This enzyme has been shown to correlate with the degree of differentiation of intestinal cells in both *in vitro* and *in vivo* models. Higher enzyme levels are found in more differentiated cell types. The present study was done to determine if enzyme levels of 13-HODE dehydrogenase are predictive of the differentiation status of biopsies from human colonic mucosa. Twenty-eight patients who underwent diagnostic colonoscopy (10 patients with adenocarcinoma and 18 with adenomatous polyps) had biopsies taken from both normal rectal mucosa and neoplastic mucosa. The determination of 13-HODE dehydrogenase activity was conducted by high-performance liquid chromatography analysis of all biopsy samples. Sixteen of the 18 patients with polyps had lower 13-HODE dehydrogenase activity in the adenoma than in the uninvolved rectal mucosa ($P = 0.001$). The colon adenocarcinomas also had less 13-HODE dehydrogenase activity in the cancer biopsy tissue than in uninvolved rectal mucosa ($P = 0.041$) These data are consistent with a role for 13-HODE dehydrogenase in intestinal cell differentiation. Understanding the precise role of this enzymatic reaction could be important potentially in the therapy and biology of colon cancer. In addition, measurements of 13-HODE dehydrogenase may be a useful parameter by which to ascertain the differentiation status of intestinal cells *in vitro*.

Introduction

Recent studies have shown that linoleic acid, which is a major dietary polyunsaturated fatty acid, is metabolized to oxidized derivatives in a number of cell types. More importantly, these oxidized derivatives have been shown to be involved in numerous cellular regulatory processes (1-5). These compounds will alter cellular function when applied exogenously to biological systems and have also been shown to be produced in response to a variety of stimuli. Therefore, both the metabolic products and the enzymes involved in their production are potential sites for therapeutic intervention.

The oxidative metabolism of linoleic acid involves the enzymatic conversion of linoleic acid into hydroperoxy, hydroxy, and keto-fatty acids (6, 7). Major metabolites are formed at either the 9- or 13-position of the polyunsaturated fatty acid. A pathway depicting metabolism at the 13-position is summarized in Fig. 1. The reactions are initiated by formation of the hydroperoxide 13-hydroperoxyoctadecadienoic acid due to the action of lipoxygenase or cyclooxygenase enzymes. The particular enzyme involved is dependent on the tissue under observation (8, 9). The second reaction in the pathway is peroxidase-mediated reduction to 13-HODE.[3] The final reaction is dehydrogenation of 13-HODE to 13-OXO, which is catalyzed by the enzyme 13-HODE dehydrogenase. The role of this latter enzyme is the focus of the current study.

The discovery of the enzymatic conversion of 13-HODE to 13-OXO was followed by partial purification and characterization of the protein involved (7, 10). The enzyme was shown to be cytosolic with a unique inhibitor specificity. In particular, 13-HODE dehydrogenase was not inhibited by known inhibitors of either alcohol dehydrogenase or diol dehydrogenase. It is slightly inhibited by disulfiram. Studies of the substrate specificity demonstrated that the preferred substrate is indeed, 13-HODE. Therefore, it can be argued that the existence of a 13-HODE-specific dehydrogenase is consistent with the hypothesis that 13-HODE is an important regulator of cellular function. Accordingly, alterations in the systems for metabolism of 13-HODE should also affect cellular regulation.

The formation of the hydroxy fatty acid 13-HODE has been reported in numerous cell types from both human and animal sources. For example, 13-HODE has been detected in human leukocytes, human platelets, bovine and rat blood vessels, guinea pig pulmonary macrophages, tracheal epithelial cells, and several cultured cell lines (2-4, 8, 9, 11-15). The compound is produced in response to physiological stimuli and also modifies cellular responses when applied exogenously. The cellular activities modified include, among others, response to growth factors, cell-cell interactions, and second messenger production (1-4, 16-19). The fact that the effective concentration of 13-HODE can be in the nanomolar range, in addition to the tissue-specific nature of responses, raises the possibility that receptors are involved.

In addition to the studies in tissues mentioned above, 13-HODE dehydrogenase has also been suggested to play an

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[3] The abbreviations used are: 13-HODE, 13-hydroxyoctadecadienoic acid; 13-OXO, 13-oxooctadecadienoic acid.

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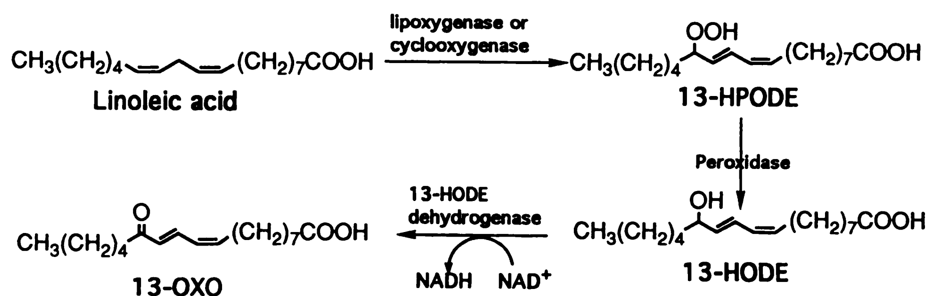

Fig. 1. Oxidative metabolism of linoleic acid to 13-HODE and 13-OXO.

important role in gastrointestinal cellular physiology. High levels of the enzyme are found in the small intestine, liver, and colon, relative to most other tissues assayed (7). In addition, the level of activity shows a strong correlation with the degree of differentiation of intestinal mucosal cells. Cells in the proliferative zone have relatively low levels of 13-HODE dehydrogenase, whereas differentiated cells near the tops of the villi or crypts have high levels of enzyme activity (20). In addition, *in vitro* investigations have shown that 13-HODE dehydrogenase activity correlates with the differentiation status of malignant colorectal cells. In both HT-29 and Caco-2 cells, 13-HODE dehydrogenase activity is higher in cells with a more differentiated phenotype (21).

The fact that the metabolite 13-HODE is produced by proliferating cells, whereas the enzyme 13-HODE dehydrogenase is elevated in differentiated cells, raises the possibility that the enzyme functions as part of a switch between proliferative stimuli (13-HODE formation) and cellular differentiation (13-OXO formation). We wished to further test the hypothesis that the enzyme is associated with the differentiation status of intestinal cells by comparing enzyme levels in normal rectal mucosa with those in neoplastic tissue from the same patient. One would expect that if 13-HODE dehydrogenase is associated with differentiation, there would be higher levels of activity in normal mucosa relative to the levels in neoplastic tissue.

Materials and Methods

Sample Acquisition. Twenty-eight patients undergoing diagnostic colonoscopy were included in the study. Ten patients had adenocarcinoma of the colon, whereas 18 patient had adenomatous polyps. Biopsies were obtained from the neoplasm as well as the uninvolved rectal mucosa. Tumors were at least 10 cm from the rectal biopsy. One to two pinch biopsies were taken from each site. The samples were frozen immediately in a dry ice/methanol bath and stored at $-80°C$ until enzyme activity was assayed, which was always within 30 days of collection. The protocol was approved by the Human Investigation Committee of William Beaumont Hospital (Royal Oak, MI).

Patient Population. The age range of the adenocarcinoma patients was 36–89 years, with a mean age of 65.8 years. The patients were prepped with either Golytely (seven patients) or Fleet's Phospho-soda (three patients). The age range of those with adenomatous polyps was 36–83 years, with a mean age of 65.9 years. These patients were also prepped with either Golytely (12 patients) or Fleet's Phospho-soda (6 patients). One individual in this group was found to have two polyps, one tubulovillous and the other tubular. Biopsies were obtained from both polyps.

Assay and Statistical Methods. The activity of 13-HODE dehydrogenase was determined by high-performance liquid chromatograpy analysis as described previously (22). Briefly, the cell pellet was sonicated in a buffer containing 20 mM potassium phosphate (pH 7.4), 1 mM DTT, and 0.1 mM EDTA. The homogenate was clarified by brief centrifugation, and enzyme activity in the supernatant fraction was determined. Protein concentration is determined by the method of Bradford (23). Incubations, in a total volume of 1.0 ml, contain 400 μg protein, 0.4 mM NAD^+, and 0.2 mM 13-HODE. After a 60-min incubation at 37°C, the samples were extracted with ether, and the extracts were analyzed by straight-phase high-performance liquid chromatograpy. The formation of 13-OXO in the sample was determined by comparison to a standard curve. Identical incubations in the absence of enzyme were used as blanks and routinely showed negligible formation of 13-OXO. Enzyme activity is expressed as pmol/mg/min. The paired t test was used for statistical comparisons between data sets.

Results

The mean level of 13-HODE dehydrogenase activity in the biopsy samples is comparable to levels found previously in animal tissues and cultured cell lines (20, 21). In general, our experience with this assay indicates that levels of activity above ~20 pmol/mg/min can be determined reliably, whereas values below this limit are somewhat less reproducible. In some cases this is a function of the amount of protein in each incubation. Although amounts of 400 μg/incubation are achieved routinely, in some cases smaller sample sizes lead to smaller amounts of protein in the incubations. In these cases the SD of the data set is often relatively large (>25% of the mean). Sample sizes of <100 μg cannot be reliably quantitated and are not included in the data presented. Thus, the data presented represent assays under optimal conditions in which the sample size and handling do not adversely affect the results.

The 13-HODE dehydrogenase activity in uninvolved rectal mucosa and colon adenocarcinoma from the same patient is presented in Table 1. In nine of ten patients, the uninvolved rectal mucosa had higher 13-HODE dehydrogenase activity than in the adenocarcinoma. The mean activity in rectal mucosa is 1.9-fold higher than the mean activity in cancer tissue. The paired t test indicates that the difference in enzyme activity between the uninvolved rectal mucosa and the adenocarcinoma tissue is statistically significant ($P < 0.041$). Although the difference in activity between tissue samples in a single individual is highly predictable, the absolute value of activity does not appear to be predictive of the differentiation status of the tissue sample in and of itself. For example, in several cases uninvolved rectal mucosa in one individual had lower enzyme

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Table 1 13-HODE dehydrogenase in cancer tissue and uninvolved mucosa of the same individual[a]

Patient	Flat mucosa	Cancer
S. C.	25.9 ± 0	7.3 ± 1.3
H. K.	104.7 ± 69.1	60.9 ± 14.9
E. K.	132 ± 4	235 ± 4
P. N.	201.5 ± 13.1	140.3 ± 3.8
G. W.	96.8 ± 7.6	7.8 ± 3.6
A. W.	164 ± 0	40 ± 4
G. W.	70 ± 1	21 ± 0
R. W.	184.4 ± 1	83.3 ± 11
K. W.	44 ± 4	0.0 ± 0
P. H.	414.8 ± 28.4	152.2 ± 18
Mean	143.8 ± 111	74.8 ± 78.1

[a] Data represent the mean ± SD of duplicate incubations.

Table 2 13-HODE dehydrogenase activity in colonic mucosa and polyps from the same individual

Patient	Flat mucosa	Polyp
Tubular		
D. J.	81.1 ± 22	ND[a]
T. L.[b]	265.7 ± 31.2	8.3 ± 0.2
D. L.	353.3 ± 2.5	31.0 ± 1.1
W. W.	19.6 ± 17.4	ND
J. A.	263 ± 7	117 ± 7
G. G.	223 ± 52.2	103.9 ± 16.5
Tubulovillous		
P. M.	ND	1.3 ± 0.8
T. L.	265.7 ± 31.2	ND
B. P.	46.6 ± 7.9	20 ± 28.3
G. W.[c]	100.4 ± 15.8	44.2 ± 6.5
R. E.	215.2 ± 37.1	204.8 ± 1.9
S. M.	65 ± 30.2	14 ± 9.6
R. S.	235.9 ± 186.7	300.4 ± 89.6
L. V.	169 ± 0	24.8 ± 1.6
M. W.	160.2 ± 7.9	75 ± 5.8
J. W.	372.4 ± 89.4	80.2 ± 1.8
H. N.	77 ± 1	70 ± 6
Villous		
E. C.	173 ± 1	104 ± 4
All polyps		
Mean	165.2 ± 108.7	70.1 ± 78.7

[a] ND, not detectable.
[b] Patient had two polyps, tubular and tubulovillous; only one was used for statistical analysis.
[c] Rectal polyp adjacent to normal mucosal biopsy site.

activity than cancer tissue in a different patient. On the other hand, the enzyme activity in an individual's cancer was almost always less than in the uninvolved mucosa regardless of the level of enzyme activity measured.

Similar results were found when the activity of 13-HODE dehydrogenase was compared between rectal mucosa and adenomatous polyps in the same patient. These data are presented in Table 2. The polyps were classified according to standard histological criteria as tubular, tubulovillous, and villous adenomas. In one patient, two separate polyps were biopsied for determination of 13-HODE dehydrogenase activity. However, for statistical purposes only one polyp was included in the analysis. In 16 of 18 samples, the enzyme activity was higher in normal mucosa than in the polyp from the same patient. As before, the paired t test indicated that the difference was statistically significant ($P < 0.001$). The mean value for 13-HODE dehydrogenase in the normal mucosa is 2.4-fold over the activity in adenomatous tissue. However, as with the cancer tissue, individual 13-HODE dehydrogenase activities were not predictive of the differentiation status in a single tissue sample.

Discussion

There is mounting evidence that the oxidative metabolism of linoleic acid plays a role in cellular regulation. Recent experiments have suggested that secondary metabolites of the primary oxygenation products play a role in both cellular proliferation and differentiation (3–5, 20, 21). Clearly, such a role would have important consequences for the development of intestinal polyps and cancers. In the present study, we have determined the levels of 13-HODE dehydrogenase, a key enzyme in the metabolism of oxidized linoleic acid, in human colon biopsies from both neoplastic colon tissue and uninvolved rectal mucosa within the same individual.

In previous experiments, the activity of 13-HODE dehydrogenase was found to correlate with the differentiation status of intestinal tissue (20, 21). The correlation was found both *in vivo*, in rat intestine, and *in vitro*, in cultured colorectal cell lines. For example, in rat intestinal mucosa, both colon and small intestine, the activity of 13-HODE dehydrogenase was significantly higher in the differentiated region at the top of the crypts or villi than in the proliferative zone near the base (20). The enzyme activity was shown to increase in concert with other biochemical markers of differentiation. Similar correlations with differentiation were observed in colorectal cancer cell lines. In spontaneous (Caco-2 cells) as well as induced (HT-29 cells) models of intestinal cellular differentiation the level of 13-HODE dehydrogenase increased as the cells became more differentiated (21).

To further examine these observations, we set out to determine if similar correlations of 13-HODE dehydrogenase with cell differentiation could be seen in colon mucosa in patients with adenomatous polyps and adenocarcinomas. If 13-HODE dehydrogenase activity plays a role in the determination of cellular differentiation then one would predict that enzyme activity would be highest in the more differentiated tissue. As shown by our data, this is indeed the case, in fact in 25 of 28 patients, the level of activity in the uninvolved mucosa was significantly higher than that in the neoplastic tissue from the same individual. Thus, these data are consistent with the hypothesis that 13-HODE dehydrogenase plays a role in the differentiation of intestinal mucosa.

Given that 13-HODE dehydrogenase activity is a potential marker of cellular differentiation, it is reasonable to suggest the enzyme may have value as a tool for evaluation of malignant potential. However, based on our results, although the enzyme activity within an individual is indicative of the differentiation status of a particular biopsy specimen, the utility of the absolute value of enzyme activity in predicting the neoplastic potential of a single sample is poor. There are a number of potential reasons for this that need to be examined in additional experiments. For example, the method of bowel preparation before biopsy, recent dietary history, medications the patient may be taking, and biological factors such as circadian rhythms may influence 13-HODE dehydrogenase activity. The cellular composition of the biopsy sample itself may also be a factor because colon biopsy specimens include cells other than colonocytes.

An additional consideration concerns the potential for zone-dependent changes in enzyme activity throughout the colon. In the present study, we have compared rectal mucosa to

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neoplastic tissue. Although we did not perform an extensive study of the distribution of enzyme activity in the various regions of the bowel, we saw no consistent variation in activity in neoplastic tissue taken from different regions of the bowel. In addition, we have recently examined the level of 13-HODE dehydrogenase in surgically obtained specimens, and the data show similar differences between tumor tissue and uninvolved mucosa, and no region-specific changes in activity are noted.[4] Therefore, we are confident that the differences observed in the present study represent true differences between normal and neoplastic tissues.

The difference in enzyme activity between normal-appearing mucosa and tumor tissue is between 2–3-fold in most of the specimens examined. Although this is a rather modest difference, the consistency of the observation (in the vast majority of samples) enhances the significance of the data. In addition, this difference is similar in magnitude to those observed in animal and cell culture studies (20, 21). Because the precise role of 13-HODE dehydrogenase in cellular differentiation has not yet been defined, it is also not clear how great a change in enzyme activity is necessary to affect cellular function. Also, there does not appear to be a significant difference in activity between cancer and polyp tissue; thus, the argument can be made that changes in 13-HODE dehydrogenase represent an early alteration in neoplastic progression. In addition, the possibility that decreased enzyme activity in association with neoplasia represents an epiphenomenon cannot, at present, be entirely discounted.

In summary, although the level of 13-HODE dehydrogenase activity from a single individual is higher in uninvolved rectal mucosa compared with neoplastic tissue, the absolute enzyme activity is highly variable from person to person. In general, individuals with low activity tended to have low activity in both tissue samples and *vice versa*. Whether 13-HODE dehydrogenase ultimately becomes clinically useful as a diagnostic, prognostic, or therapeutic target remains to be determined. However, an alternative use of the measurement of 13-HODE dehydrogenase activity may be in assessing the determination of the differentiation status of intestinal cells *in vitro*. In any case, unraveling the biology of linoleic acid metabolism in the intestine offers promise for gaining insight into potential methods for the therapy and chemoprevention of colorectal neoplasms.

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