Histochemical detection of lipid peroxidation in human gastrointestinal, mammary and renal carcinomas.

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Abstract

Constitutional lipid peroxidation in randomly selected 32 cases of clinically advanced carcinoma from human gastrointestinal tract (20 cases), breast (8 cases) and kidney (4 cases) was examined histochemically in frozen sections using cold Schiff’s reagent. Only two cases of gastrointestinal carcinoma were positive by the reagent. Non-cancerous parenchymal cells were negative. These findings suggest that detectable constitutional lipid peroxidation seldom occurs in either cancerous or normal tissues. The capacity for normal and neoplastic tissues to undergo lipid peroxidation was also studied by incubation with an iron-NADPH pro-oxidant system. Normal parenchymal cells showed, to various degrees, a positive reactivity. In gastrointestinal carcinoma, 6 out of 7 cases of well differentiated adenocarcinoma reacted positively, whereas 2 out of 8 cases of moderately to poorly differentiated adenocarcinoma disclosed weakly positive reactions. Mucinous adenocarcinomas (4 cases) were all negative. Signet-ring cell carcinoma (1 case) was positive. One out of 8 cases of breast cancer also showed positive reaction. Four renal cell carcinomas were all negative. Cancer cells have lower capacity to undergo lipid peroxidation than normal cells, when the iron-NADPH pro-oxidant system was employed. In gastrointestinal carcinoma, the ability to undergo lipid peroxidation by the iron-NADPH pro-oxidant seems to be correlated with their histological differentiation. This fact may suggest that differences in lipid composition or the NADPH enzyme system exist between well differentiated and poorly differentiated gastrointestinal malignancies.

KEYWORDS: lipid peroxidation, histochemistry, cancer, iron, NADPH

*PMID: 1442147 [PubMed - indexed for MEDLINE]
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Histochemical Detection of Lipid Peroxidation in Human Gastrointestinal, Mammary and Renal Carcinomas

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Lipid peroxidation of the biological membrane by free radicals is thought to induce tissue injuries (1,2), neoplastic transformation (3-6), and aging (7). A recent study showed that repeated induction of lipid peroxidation by multiple injections of ferric nitrotriacetic acid in the mice caused renal cell carcinoma (8). It is interesting to note, however, that neoplastic cells themselves show decreased lipid peroxidation under the oxidative stress, when compared with normal cells (9-11). Chemically induced pre-neoplastic lesion of the colon also shows a decreased capacity to undergo lipid peroxidation (12). On the other hand, the increase in thiobarbituric acid-reactive substances of lipid peroxidation has been reported in human colorectal carcinoma (13). Therefore, the liability

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of neoplastic tissues to lipid peroxidation is still obscure. In this paper, we studied the constitutional lipid peroxidation as well as the capacity to undergo lipid peroxidation by iron-NADPH in 32 advanced human cancers. A histochemical method was used in order to identify cells responsible for lipid peroxidation.

Materials and Methods

Patients. Thirty-two patients (14 men and 18 women) with clinically advanced carcinoma of gastrointestinal tract (20 cases), breast (8 cases) and kidney (4 cases) were studied. The age of the patients ranged from 24-81 years (median, 62 years). Additional details of the patients are presented in Table 1.

Reagents. Basic fuchsin was obtained from EM Science (NJ, USA). \( \beta \)-NADPH was from Oriental Yeast Co., Ltd. (Tokyo, Japan). OCT tissue embedding compound was from Miles Inc. (IN, USA). Other reagents were of the highest quality available.

Specimen handling. Neoplastic and non-neoplastic tissues were obtained from the same organ by surgical resection. Tissue samples were embedded in OCT compound, frozen immediately, and stored at \(-40^\circ\text{C}\). Histochemical examinations were carried out within 3 days.

Histochemical detection of lipid peroxidation. Histochemical detection of lipid peroxidation was performed by the method of Pomppella et al. (14), using cold Schiff reagents (15). The specificity of this method has been well studied by Benedetti et al. (9), and this method detects protein-bound aldehydes as well as carbonyl functions present in acyl residues of peroxidized phospholipids of cellular membrane. Normal elastic fibers of blood vessels and connective tissue were also stained by this method irrespectively of lipid peroxidation (16). Frozen sections (10-15 \( \mu \text{m}\) thick) were stained for 2 h in the dark at room temperature with cold Schiff’s reagent. After the reaction, the sections were rinsed in three changes of sulfite water (0.5% \( \text{K}_2\text{S}_2\text{O}_5\) containing 0.05N HCl), and then counterstained with Meyer’s hematoxylin. The areas of lipid peroxidation were stained purple.

Induction of lipid peroxidation in vitro on frozen sections. To determine the capability of tissues to undergo lipid peroxidation in the presence of ferric nitroprussiactate-NADPH peroxidant system, their serial frozen sections (10-15 \( \mu \text{m}\) thick) were incubated at room temperature for 30 min at pH 7.4 in 150mM NaCl, and 10 mM Hepes buffer which contained 0.5mM NADPH and 0.5mM iron. The iron solution was prepared by mixing \( \text{FeCl}_3\) and nitroprussiactate to a molar ratio of 1:3 by the method of Awai et al. (17). After incubation, the sections were rinsed and stained with Schiff’s reagent to detect lipid peroxidation as described above.

Results

Constitutional Lipid Peroxidation

Gastrointestinal tract. Among the 20 cases examined, normal epithelial cells in all cases and cancer cells in 18 cases were completely negative for the Schiff reaction. Certain parts of the cancer tissue showed positive reaction in two cases, one was a well differentiated adenocarcinoma of the stomach (Fig. 1a, 1b), and the other was a mucinous carcinoma of the colon (Fig. 2a, 2b). In the case of gastric carcinoma, the apical membrane of the cancer cells showed a positive reaction (Fig. 1b). In such areas, an accumulation of neutrophils, macrophages and necrotic cells were observed in the neoplastic gland (Fig. 1a). In the colon cancer, mucin lakes
were surrounded by tall columnar cancer cells (Fig. 2a), the apical membrane of which reacted positively, (Fig. 2b). Inflammatory cells were not apparent in this colon carcinoma.

**Breast.** Eight specimens were examined. Both the cancer tissue and the normal ductal epithelium were negative for Schiff’s reagent.

**Kidney.** Four specimens were examined. A positive reaction was not observed in either carcinoma or non-neoplastic cortical tissue.

**In Vitro Tissue Liability to Lipid Peroxidation by iron-NADPH Oxidative Stress**

**Gastrointestinal tract.** Normal epithelial cells showed various degrees of positive reaction. In the stomach, parietal cells of the fundic gland showed markedly strong staining (Fig. 3). The reaction was generally weaker and focal in the neoplastic cells when compared with the corresponding normal epithelial cells, and the ability to undergo lipid peroxidation of the cancer cells seemed to correlate with the degree of their histological differentiation. The results are summarized in Table 2. Well differentiated adenocarcinomas showed spotted or patchy positive reaction in 6 out of 7 cases (Fig. 4), whereas moderately to poorly differentiated adenocarcinomas showed weak reaction in 2 out of 8 cases. Mucinous adenocarcinomas (4 cases) were negative. The signet-ring cell carcinoma (1 case) was positive. Smooth muscle cells and lymphocytes also showed positive reaction. The interstitial tissue showed focal positive reaction.

**Breast.** Normal ductal epithelial cells showed positive reaction. Among 8 cases of carcinoma, only one case of scirrhous carcinoma reacted positively (Fig. 5). Normal adipose tissue was not peroxidized.

**Kidney.** Proximal tubules in the renal cortex

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**Fig. 1a** Well differentiated adenocarcinoma of the stomach. Note that neutrophils, macrophages and necrotic cells accumulate in the neoplastic gland. Formalin-fixed paraflin-embedded section. HE × 180

**Fig. 1b** Cold Schiff staining of the same case. Apical membrane of the cancer cells shows positive reaction. Inflammatory cells in the neoplastic gland also show positive reaction. × 180
**Fig. 2a**  Mucinous adenocarcinoma of the colon. Formalin-fixed paraffin-embedded section. HE $\times$ 180

**Fig. 2b**  Cold Schiff staining of the same case. Part of the apical membrane is stained by Schiff’s reagent. $\times$ 180

**Fig. 3**  Cold Schiff reaction of the normal gastric mucosa after incubation with iron-NADPH system. Parietal cells of the fundic gland are more strongly stained than other epithelial cells. $\times$ 180

**Fig. 4**  Cold Schiff reaction of the well differentiated adenocarcinoma of the colon after incubation with iron-NADPH system. Some cancer cells show positive reaction at plasma membrane and cytoplasm. $\times$ 90
carcinomas of the gastrointestinal tract, breast and kidney were studied histochemically for constitutional lipid peroxidation and iron-NADPH-induced \textit{in vitro} lipid peroxidation. Normal parenchymal cells were completely negative for constitutional lipid peroxidation. Only two cases of gastric and colon cancer showed detectable amounts of lipid peroxidation. This observation suggests that lipid peroxidation of the parenchymal cells seldom occurs in living organisms.

Table 2  Reaction of gastrointestinal adenocarcinomas detected by iron-NADPH system

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>Number of patients$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated adenocarcinoma</td>
<td>7 (6)</td>
</tr>
<tr>
<td>Moderately differentiated adenocarcinoma</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Poorly differentiated adenocarcinoma</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>4 (0)</td>
</tr>
<tr>
<td>Signet-ring cell carcinoma</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

$^a$: Figures in parentheses indicate the number of positive cases.

Fig. 5  Cold Schiff reaction of the scirrhus carcinoma of the breast after incubation with iron-NADPH system. Cancer cells show positive reaction. \( \times 180 \)

Fig. 6  Cold Schiff reaction of the kidney after incubation with iron-NADPH system. Proximal tubules show positive reaction, whereas a glomerulus is not stained. \( \times 180 \)

were markedly peroxidized; however, glomeruli, medulla, and distal tubules were hardly peroxidized (Fig. 6). Four cases of renal cell carcinoma showed no detectable lipid peroxidation by the iron-NADPH system.

Discussion

In this paper 32 cases of clinically advanced under normal conditions at a histochemically detectable level. In one case of gastric carcinoma, the positive Schiff reaction of the cancer cells were observed near the accumulation of neutrophils and macrophages. NADPH oxidase is strongly active in activated neutrophils which neutrophils are potent generators of active oxygen species (18). Therefore, the lipid peroxidation of the cancer may have been induced by these neutrophils \textit{in vivo}. Increased thiobarbituric acid-
reactive substance with coincidental increases in phospholipase A2 and myeloperoxidase have been reported in human colorectal carcinoma (13). In these cases, inflammatory cells may be the cause of elevated lipid peroxidation. Activated neutrophils may also induce lipid peroxidation in non-neoplastic cells. However, marked accumulation of the neutrophils was not observed in non-neoplastic tissues in our specimens, and therefore the effects of neutrophils on non-neoplastic cells are still obscure.

Many recent reports have suggested that neoplastic cells lose the ability to undergo lipid peroxidation (9–11). In this study we used ferric nitrolotriacetate-NADPH to induce lipid peroxidation (19). Although the precise mechanism is still obscure, a weakly-chelated iron complex can effectively induce lipid peroxidation. NADPH is required to reduce ferric to ferrous iron non-enzymatically, or this reaction may be mediated by microsomal enzyme (20). In our experiments, parietal cells of the stomach and proximal renal tubular epithelium were well peroxidized by iron-NADPH. Other epithelial cells showed lower peroxidation. It is suggested that different cells have different capacities to undergo lipid peroxidation even in normal tissue.

Gastrointestinal carcinomas were more weakly peroxidized than the surrounding normal epithelium, and well differentiated carcinomas seemed to be more easily peroxidized than moderately or poorly differentiated carcinomas. In animal neoplasms, Yoshida hepatoma cells has much lower activity for NADPH-cytochrome C reductase and NADPH-cytochrome P450 (11), both of which are involved in certain types of lipid peroxidation. Neoplastic cells, especially poorly differentiated cells may have lower activity for NADPH-dependent oxidoreductases. However, another explanation is also possible. Tumor cells may have a lower content of unsaturated fatty acid than normal cells. Indeed the fatty acid composition in several tumor cells is different from that in normal cells (11, 21). The importance of the role of α-tocopherol content in the protection against iron-induced lipid peroxidation in Yoshida hepatoma cells has been suggested (11). These tumor cells have an increased content of α-tocopherol, which seems to be critical for their decreased capacity to undergo lipid peroxidation (11). Therefore, the mechanism of decreased capacity to undergo lipid peroxidation in human cancers must be studied further.

References


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Received January 23 1992; accepted February 19, 1992.