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Peroxidative processes in human stomach adenocarcinoma

Procesy peroksydacyjne w gruczolakorakach żołądka u człowieka

It is well known that peroxidation is low in most tumors (1, 14). It seems interesting to investigate if low lipid peroxidation in tumors is a phenomenon related to the neoplastic state itself, or if it represents a feature accompanying dedifferentiation. Preliminary results strongly support the latter hypothesis (8, 16). Tumor cells and its organelles ofter have an abnormal lipid composition, unsually a decreased phospholipid content and a low level of polyunsaturated fatty acids (9, 15). Tumor cells also generally contain low, often undetectable levels of the enzymes of cytochrome P-450 system wich can initiate and propagate lipid peroxidation (17). In addition, related observations have been done in dividing normal cells (4).

Products of lipid peroxidation include hydroperoxides, conjugated dienes, malonaldehyde/malondialdehyde and fluorochromic proteins or amino-containing phospholipids that develop intra-or intermolecular cross-links with malonaldehyde or malondialdehyde and demonstrate features of conjugated Schiff bases (7,19).

The aim of this study was to investigitate the aldehyde pattern produced during lipid peroxidation in stomach tumors. These investigations seemed necessary due to the demonstration (9) that the intensity of the colour developed by tissue homogenates in the presence of thiobarbituric acid mostly relates to their content. During lipid peroxidation, however, a number of different aldehydes is produced, some being more toxic and more abundant than malondialdehyde. Thus, a low thiobarbituric acid values (TBA-values) as reported by most authors studying lipid peroxidation in tumors, may by only a mere consequence of different peroxidation kinetics, with less malondialdehyde produced, and independent of the real amount of other aldehydes.

MATERIAL AND METHODS

Human stomach adenocarcinomatous tumors were obtained during surgery. The diagnosis of each case was confirmed histologically in Department of Pathology, Medical Academy in Lublin. Portions of normal-appearing tissues were obtained for control study. Tissues were homogenized in 0.1 M Tris

- Hcl buffer (pH = 7.2) in a Waring Blendor homogenizer and then a 10% homogenate was centrifuged at 3,000 g for 15 min.

A suitable amount of supernatant was incubated in 37°C with or without peroxidation-initiating system. Two systems were used in which peroxidation was stimulated by ADP/Fe which requires NADPH: cytochrome c reductase (11) and ascorbate/Fe which induce lipid peroxidation by the nonenzymatic way (5).

To study the aldehyde pattern, the sample was treated with dinitrophenylhydrazine in 1 M HCl (2:1) for 2 hr in the darkness at 25°C and subsequently for 1 hr in a freezer. The pellet and supernatant were separated by centrifugation at, 3,000 g for 10 min and the pellet was extracted with chloroform-methonal mixture (21) two times. Supernatant was extracted with CH3Cl two times and the two extracts were mixed and the mixture was keept for one night in a freezer and then evaporated to dryness.

Thin-layer chromatography was carried out on silica gel G precoated plates (E. Merck, Darmstadt, Germany) in CH3Cl. As a result the three fractions were obtained: I - (polar fraction) containing OH-alkanals, OH-alkenals and the excess of dinitrophenylhydrazine; II - osazones and III - alkanals, alkenals and ketones. Carbonyl-containing band was scrapped off the plate and the gel extracted twice with CH3Cl and filtered. The carbonyl content within the unpolar fractions was calculated by UV - VIS spectra in CH3Cl using the extinction coefficient of 26.000, (wave length 353 nm).

Conjugated dienes were extracted from tissue using a 2 : 1 (vol/vol) mixture of chloroform and methonal with prior adding of 0.8 ml distilled water for each 1 g of the tissue. The material was then homogenized. Subsequently for 1 g of tissue wet weight, 6.0 ml of chloroform/methonal (1 : 2 vol/vol) were added followed by agitation for 2 min with a vortex mixter. Then, 2.0 ml of this mixture (per 1.0 g of wet tissue) were added, followed by the use of the vortex mixter for 30 sec. 2.0 ml of acidified water (pH = 2.0) were subsequently added (per 1 g of wet tissue) followed by mixing with a vortex for another 30 sec. Finally, the material was cleared by centrifugation and the chloroform layer was aspirated, transferred to a test tube and dried under a flow nitrogen. The residue was reconstituted with 0.5 of heptane and measured spectrophotometrically at 233 nm.

Lipid hydroperoxides were measured according to the iodometric procedure (3). Briefly, 1.0 ml of sample (homogenate) was mixed with 7.0 ml of chloroform/methonal (2 :1 vol/vol), agitated with a votrex mixer for 2 min and then subjected to contrifugation (1500 g) for 5 min. Aspiration of 5.0 ml of the lower chloroform layer was carried out with the chloroform extract then being dried under nitrogen flow. When dry, 1.0 ml of a acetic acid/chloroform mixture (3 : 2, vol/vol) was added, followed by 0.05 ml of potassium iodide (1.2 g/ml) an rapid shaking of the sample, which was then shielded from light for 5 min, followed by addition of 3.0 ml of cadmium acetate (0.5 g/100 ml in water). The solution was shaken vigorously, cleared by centrifugation and the upper (water) phase was aspirated and the absorbance at 353 nnm was measured. A standard curve was developed using cumene hydroperoxide (Sigma, St. Louis, USA).

Protein content was measured according to L o w r y et al. (10) with bovine serum albumin as a standard. Statistical analysis was done by computer set Statistica v. 4.0 (Statsoft Inc., Santa Clara, CA, USA). All chemicals were purchased in E. Merck, Darmstadt, Germany, unless otherwise mentioned.

RESULTS

Figure 1 shows the production of three different fractions of nonpolar cabonyls as shown by samples treated with ascorbate or ADP/Fe. The decreased response of malignant tissue is evident.

Table 1. Non-polar and medium polar carbonyls (nmole/mg protein) produced after 60 min incubation at 37°C of homogenates from normal and adenocarcinomatous stomach tissue, without 500 µmole ascorbate or 250 µmole/2 ADP/Fe (final concentrations) in 0.05 mM Tris-HCl buffer (pH = 7.2)

Stimulation	Normal tissue	Adenocarcinoma
Nome		
Fraction I	0.50 ± 0.06	0.90 ± 0.07*
Fraction II	0.42 ± 0.05	1.00 ± 0.09*
Fraction III	1.25 ± 0.15	1.61 ± 0.18
500 µM ascorbate		
Fraction I	1.00 ± 0.20	2.00 ± 0.22*
Fraction II	0.80 ± 0.08	0.31 ± 0.04*
Fraction III	2.50 ± 0.30	2.54 ± 0.28
250 µmol/2 µmol ADP/Fe		
Fraction I	1.50 ± 0.18	0.80 ± 0.09*
Fraction II	2.58 ± 0.17	0.60 ± 0.07*
Fraction III	7.00 ± 0.80	1.60 ± 0.90*

I – (polar fraction) containing OH-alkanals and OH-alkenals, II – osazones and III – alkanals, alkenals and ketones. Value are mean ± S.D.* – $p < 0.05$ vs control

Table 2. Conjugated dienes (E_{233}) and hydroperoxides (E_{353}) in malignant stomach tissue (n = 16)

Stimulation	Normal tissue	Adenocarcinoma
Conjugated dienes	0.645 ± 0.050	0.250 Ě 0.030*
Hydroperoxides	0.055 ± 0.010	0.010 Ě 0.003*

Mean ± S.D.* – $p < 0.05$ vs control

Table 1 shows the non-polar and medium polar aldehyde pattern in stomach tissues. It is evident that in adenocarcinomatous tissue, both unstimulated and ascorbate-stimulated, the levels of these aldehydes are very similar. Stimulation with ADP/Fe, however, caused a twofold rise in the aldehyde production in control stomach tissue as compared to adenocarcinomatous tissue.

Table 2 shows the conjugated diene and hydroperoxide levels in stomach adenocarcinomatous tissue. The levels of these products are low in malignant tissue as compared to control.

DISCUSSION

The initial step of hydrogen abstraction resulting in the formation of a conjugated dienes is followed by the formation of a lipid peroxide and its conversion to a lipid endoperoxide. This endoperoxide can subsequently undergo reaction with a

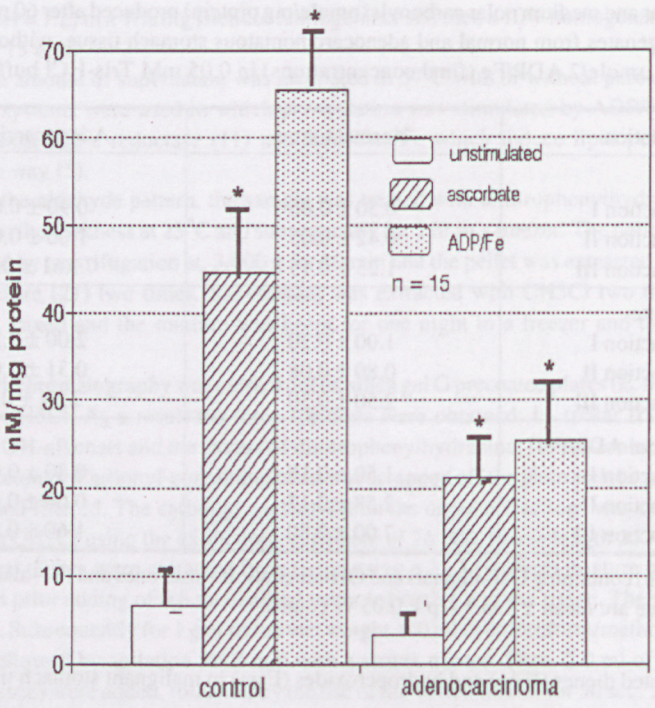

Fig. 1. MDA production in stomach. Mean ± S.D.* – $p < 0.05$ vs unstimulated

variety of compounds including hydrocarbons to form an alkyl radical and lipid hydroperoxide.

There are reasons to assume that aldehyde metabolism is very active, both through oxidative and through reductive mechanisms. Lack of lipid peroxidation in stomach adenocarcinomas is surely the consequence of several converging effects. First, the loss in activity of the metabolic chain of the smooth endoplasmenic reticulum, where the enzymatic lipid peroxidation takes place. Second, unsaturated acids are strongly decreased in tumor membranes, where they are substituted mostly by saturated acids. (Stettner and Ledwożyw, unpublished data). Third, the possibility that decreased lipid peroxidation is a consequence of the level of antixidants in tumor tissue. Experiments of C h e e s e m a n et al. (6) have shown that the content of natural lipid-soluble antioxidant, vitamin E, is strongly increased in non-perixidizing tumor cell membranes.

Superoxide dismutase (SOD, EC 1.15.1.1) catalyzes the dismutation of superoxide anion to oxygen and hydrogen peroxide while catalase (EC 1.11.1.6) catalyzes hydrogen peroxide to water and oxygen. These biological roles are thought to serve as cell defence reactions against the potentially harmful effects of superoxide anion generated by a wide variety of biological processes. Since the significance

of SOD was first described by McCord and Fridovisch (12) many experiments have been performed to determine its structure, property, mode of acions etc. (2, 21). The attention has been paid to the clinical aspects of the metabolic pathway of superoxide, especially, the relationship between cancer and the protective effects of these enzymes. In a number of cases, differences in the activity of SOD have been found between normal and cancerous cells (13), and catalase activity has shown a deficiency in cancerous cells. Saito et al. (18) have shown that the patients with malignant lymphoma and acute myeloid leukemia showed a significant decrease in both enzyme acivities, while the patients with lung cancer (squamous cell carcinomas and small cell carcinomas) showed normal value of both SOD and catalase activities. Van Balgooy and Roberts (20) also showed that malignant tissues may have lower SOD activities than normal tissue. The Ehrlich ascites and EL-4 ascites cell showed the lowest values among the mouse (lymphoid leukemia 6MP, lymphoid leukemia 5FU, lymphocytic leukemia P388, melanotic melanoma B16, Lewis lung carcinoma, lymphoid leukemia L1210/0, EL-4 ascites, Ehrlich ascites) and rat (Novikoff hepatoma, Morris hepatoma, mammary adenocarcinoma, Dunning leukemia, Walker carcinoma 256) tumor studied.

Further investigations are, however, needed to study the possible role of these enzymes in tumor cells.

CONCLUSIONS

1. The production of threedifferent fractions of nonpolar cobonyls as shown by samples trearted with ascorbate or ADP/Fe. The response of malignant tissue is evident.
2. In adenocarcinomatous tissue both unstimulated and ascorbate-stimulated the levels of aldehydes are very similar. Stimulation with ADP/Fe, however, caused twofold rise in the aldehyde production in control stomach tissue as compared to adenocarcinomatous tissue.
3. The conjugated diene hydroperoxide levels in stomach adenocarcinomatous tissue, are low in malignant tissue as compared to control.

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STRESZCZENIE

Badano poziom aldehydów, związków ze sprzężonymi dienami i hydronadtlenków w gruczolakorakach żołądka. Wykazano, że tkanka nowotworu złośliwego produkuje znacznie mniej aldehydów i produktów peroksydacji niż tkanka zdrowa.