OXIDATIVE PROTEIN DAMAGE IN PATIENTS WITH COLORECTAL CANCER

ŁUKASZ MURLIKIEWICZ, ANDRZEJ SYGUT, RADZISŁAW TRZCIŃSKI, KRZYSZTOF GRZEGORCZYK, MACIEJ RUTKOWSKI, ADAM DZIK

Department of General and Colorectal Surgery, Medical University in Łódź
Kierownik: prof. dr hab. A. Dziki

Department of Chemistry and Clinical Biochemistry, Medical University in Łódź
Kierownik: dr hab. n. biol. I. Majsterek, prof. UM

Department of Endoscopy and One Day Gastroenterology, Wł. Biegański Memorial Regional Specialistic Hospital in Łódź
Kierownik: dr n. med. K. Grzegorczyk

Colorectal cancer is a major public health concern particularly in developed countries. Despite decades of advances in the treatment and prevention of colorectal cancer, it remains the second most common cause of cancer death. There now exists convincing evidence that reactive oxygen species play an important role in the etiology and progression of a number of human diseases including colorectal cancer. Reactive oxygen species may damage all types of biological molecules. However, proteins are possibly the most immediate vehicle for inflicting oxidative damage on cells since they are often catalysts rather than stoichiometric mediators, hence, the effect of damage to one molecule is greater than stoichiometric.

The aim of the study was to investigate oxidative protein damage in patients with colorectal cancer and its correlation with the clinical stage of the disease.

Material and methods. The study group comprised 102 patients operated on for colorectal cancer in different clinical stages of the disease. Plasma carbonyl levels were determined using Levin’s method.

Results. Patients in all tumor groups showed significantly higher levels of plasma carbonyls when compared to healthy people. We observed an increase in mean plasma carbonyl levels correlating with an increase in the degree of disease advancement.

Conclusions. This study demonstrates that reactive oxygen species may have a role in pathogenesis of colorectal cancer. The outcomes of this research seem to confirm that antioxidants may play a role in chemoprevention of colorectal cancer.

Key words: colorectal cancer, carbonyl groups, oxygen reactive forms

It is assumed that in structure of morbidity and mortality present decade will no longer belong to cardiovascular disease, and the first item in this list will be cancer. According to estimates in 2050 in the United States alone, cancer incidence would be 2.6 million (1.44 million in 2008), and the mortality rate of approximately 1 million (565 thousand in 2000) (1, 2).

Also in Poland the number of registered cancers is increasing. In 2001, 86443 people died on them, and in 2005 – 90396 people. Recorded increase is much higher in men. An example of the upward trend in the incidence of cancer is colon cancer. In 1991, Poland recorded 7849 new cases of this cancer and it was the third cause of death, after lung cancer and prostate cancer in men and third after lung and breast cancer in women. However, in 1996, according to the Department of Epidemiology, Center of Oncology in Warsaw, morbidity was a total of 10 455, 7 593 deaths recorded in the course of this disease (3).
Currently, the structure of cancer cases, colorectal cancer ranks second in both sexes. Mortality from cancer of the colon among men is the second cause of cancer deaths in women—a third.

In recent years, studies show that free radical mechanisms may be responsible for initiating and developing certain cancers—including colorectal cancer (4). Reactive oxygen species can damage all cell components (proteins, lipids, carbohydrates, and nucleic acids). Especially adverse effects of this type of interactions observed in the case of proteins, because they perform a number of regulatory functions in many biochemical processes.

Under the influence of oxidants occurs in proteins to damage the structure of the protein chain, oxidative modification of amino acid residues and prosthetic groups. This may lead to rupture polypeptide chain, making cross linkages within the same chain or other chains polypeptide and changes in the structure of amino acids and components complex proteins (5).

Hydroxyl radical oxidation of the polypeptide chain initiates tearing hydrogen atom at the alpha carbon amino acid. The resulting alkyl radical reacts with oxygen to form by radical alkyl peroxide to alkyl hydroperoxide. Emerging from it alkox radical may be converted into a hydroxyl alpha carbon rest of the amino acid or can lead to fragmentation of the polypeptide chain.

Radicals: alkyl, alkoxy and alkyl hydroperoxide react with other amino acid residues the same or another polypeptide protein chain, allowing subsequent formation of radicals. Deficiency of oxygen radicals when creating alkyl hydroperoxide is difficult, alkyl radicals within the same or different proteins, reacting with each other may lead to the formation of cross linkages between the chains polypeptide.

 Interruption of polypeptide chain can occur through interaction of reactive oxygen species in the rest of glutamic acid, aspartic acid, and proline. All the rest of the amino acid occurring in proteins are susceptible to oxidation. The greatest sensitivity to the effects of reactive oxygen species are characterized by: cysteine, methionine, tyrosine, and tryptophan. The cysteine rest oxidize to disulfide residues and methionine residues to methionine sulfoxide. It is the only modification of amino acids in proteins in vivo, which can be corrected in the presence of specific reductases.

Particularly vulnerable to the adverse effects of reactive oxygen species are the rest of the aromatic amino acids. As a result of oxidation of tyrosine 3,4-dihydroxyphenylalanine arises or comes to making cross linkages between the aromatic rings of two molecules of this amino acid. The tryptophan residues oxidize to and formylkynurenin and kynurenin the histidine to 2-oxyhistidine, asparagine and aspartic acid.

Oxidation of amino acid residues with free amino group, amido or hydroxyl leads to carbonyl derivatives. Such derivatives can react with other free amino groups of lysine residues in the same or another protein molecule to form cross-bonds (5).

The reactions of reactive oxygen species with proteins leads to modification of amino acid modifications and prosthetic groups, aggregation or fragmentation of protein molecules. Typically, these changes occur simultaneously and cause a disturbance of biological activity of proteins and the creation of aggregates. Effects on protein depends on the nature of the source of reactive oxygen species and exposure conditions on them.

How much impact can bring damage to the proteins described above demonstrates the fact that the body countless biochemical reactions are catalyzed by enzymes, which are proteins (6). In addition, oxidatively damaged proteins are not removed by proteosomic systems in an efficient manner, resulting in their accumulation in the cell and impair its proper functioning. Formation and accumulation of oxidized protein products play an important role in the pathogenesis of not only cancer but also vascular and neurodegenerative.

The aim of this study was to assess the degree of oxidative damage to proteins in patients with cancer of the distal large intestine depending on the clinical stage of cancer. As a biomarker of oxidative modification of protein content used in the measurement of carbonyl groups in serum proteins.

MATERIAL AND METHODS

The study group comprised 102 patients (42 women and 60 men; average age 64.4 ± 10.9 years) operated on for colorectal cancer at the Department of General and Colorectal Surgery, Medical University in Łódź, during the period between 2006 and 2008.
The investigated patient groups, depending on the stage of the disease showed no statistically significant differences, considering average patient age and gender.

Controls consisted of 20 healthy, non-smoker volunteers with no history of previous disease. None of the investigated patients received vitamin supplements at least six months before study initiation.

All patients were referred for surgical treatment. Obtained in the course of treatment specimen was assessed histopathologically and its outcome, together with the results of radiological studies were based on the classification of patients into different groups according to clinical stage of disease with the Dukes classification. In the case of inoperable tumors, where no material was obtained for pathomorphological examination, the classification was based on a result of the histopathological examination was carried out before surgery, clinical examination, intraoperative evaluation of tumor and available imaging studies.

Plasma carbonyls level was determined using Levin’s method (7). Absorbance of the sample treated with dinitrophenylhydrazine terms of the control sample was measured at a wavelength of 370nm. Absorbance measurements were performed using the Cary 100 device (Varian 2006 EL06083835). The content of carbonyl groups was calculated based on the absorption milimol coefficient formed hydrazone 22.000 M⁻¹ x cm⁻¹.

Each sample was tested three times, and the carbonyl content expressed in nmol / mg protein is the arithmetic mean of three measurements.

RESULTS

Table 1 and fig. 1 present plasma carbonyls values in patients group depending on the stage of the disease, according to Dukes classification, as compared to the healthy control group.

We found statistically significant (p<0.05) higher plasma carbonyls values in all stages of the neoplastic disease, as compared to the control group (p<0.05).

Additionally, analysis comprised the comparison between results obtained from particular disease stage groups. We observed an increase in mean plasma carbonyl levels correlating with an increase in the degree of disease advancement. In all cases differences were statistically significant (p<0.05).

DISCUSSION

In the seventies the eighteenth century, Carl Sheela and Joseph Priestley described the life-giving effects of oxygen on living organisms. The first question of the harmfulness of certain forms of this element emerged with the discovery of anaerobic bacteria, and the first response was given in 1978 by Irvin Fridovich. He published the so-called. „Superoxide theory of oxygen toxicity.” Effect of reactive oxygen species in the individual components of the body’s building elements have been investigated relatively well, especially when it comes to oxidative modification of lipids (8). The products of these transformations, especially dialdehyde malonic (MDA), are used as indicators of oxidative stress the body.

Lipid peroxidation is a chain reaction, free radical oxidation process. Modification of the rest are mainly polyunsaturated fatty acids incorporated in phospholipids, which are the main element of cell membranes. MDA – one of the best-studied products of lipid peroxidation, modifies the physical properties of cell membranes, and the effect of this distortion are hydrophobic interior of lipid membranes and the infringement of its two-layer structure, which leads to disturbances of its normal function and impairment of individual cells, and ultimately the entire organ.

During the analysis of these findings raises the question of what will be effects of reactive oxygen species on other components of the body’s building elements, especially for proteins – compounds involved in the numerous biochemical processes in the body, even in the form of enzymes?

Stadtman et Bedett postulate that the oxidative damage of proteins may be in vivo more important than damage to lipids (10). In assessing the effects of reactive oxygen species on protein measurement of carbonyl groups content is used. Dalle-Donne et al. present the advantages of this method in comparison with other free radical reactions products (6). In general, the formation of protein carbonyl derivatives occurs early in the course of these processes than other products of oxidation, and the resulting derivatives are relatively stable compounds, which
allows for easier determination of the parameters and safe storage of samples without the risk of adverse effects on the measurement results obtained.

Carbonyl derivatives can be produced not only by direct effects of reactive oxygen species on the protein, but also as a result of secondary effects of lipid peroxidation products or carbohydrates on the nucleophilic side chains of certain amino acids. Although whether the carbonyl groups arising from direct or indirect modification of amino acids, additional tests are needed, a measure of content of carbonyl groups in proteins is now recognized as the best and, as yet, the most commonly used indicator of overall oxidative modification of proteins (10, 11, 12).

Participation of reactive oxygen species is postulated in the formation and development of numerous diseases, including cancer (6, 10, 11, 13, 14). Colorectal adenocarcinoma is the most common gastrointestinal tract malignancy. Is a serious and ever-increasing health problem worldwide. That’s why we decided to conduct this research among patients diagnosed with distal colon cancer (sigmoid colon and rectum), assessing in this group of patients the effects of reactive oxygen species in the main building elements of the body, such as proteins.

One of the first studies to evaluate the content of carbonyl groups in serum proteins in patients with cancer were conducted in 1998 by a team of Polish researchers – Popadiuk et al. In 60 children diagnosed with cancer, they found two times higher content of carbonyl groups in serum proteins compared with healthy children (15). Similar results were obtained by Pignatelli et al. for patients with lung cancer. In the group of 52 patients found significantly higher plasma concentrations of proteins containing carbonyl groups than in healthy subjects (16). Yilmaz et al. studied the oxidation parameters of proteins in 43 patients with bladder cancer and compared them to a group of 28 healthy volunteers (17). In patients with cancer found a statistically higher content of carbonyl groups as compared with the control group. Yilmaz made the analysis of the parameters depending on the stage of the disease, finding higher values in patients with invasive tumors (T2–T4) than the non-invasive (T1), although in this case the differences were not statistically significant.

The measurements we conducted of the content of carbonyl groups in serum proteins in patients with cancer of the rectum and sigmoid colon also gave statistically significantly higher in comparison with healthy subjects. With respect to the control group noted a higher content of carbonyl groups in each stage of the disease.

Similar results were obtained by Chang et al. noting among 36 patients with colorectal cancer increased content of carbonyl groups in serum proteins while reducing the activity of antioxidant enzymes and concentration of vitamins C and E in plasma. In the study group, malondialdehyde concentration in plasma was lower than in the control group. The result of this study suggests the conclusion that the determination of carbonyl groups may be more sensitive parameter of the impact assessment of oxidative damage in the body (19).

<table>
<thead>
<tr>
<th>N=O</th>
<th>Dukes classification</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Number of patients</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>Median</td>
<td>0,583</td>
<td>0,696</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0,105</td>
<td>0,191</td>
</tr>
<tr>
<td>Arithmetical mean</td>
<td>0,570</td>
<td>0,689</td>
</tr>
</tbody>
</table>

Fig. 1. Mean and standard deviation of plasma carbonyls values depending on the stage of the neoplasm, according to Dukes classification (* p<0.05)
Drew et al. (18) attempted to assess oxidative damage to cellular proteins in intestinal pre-cancerous lesions in mice. Deregulation of the functioning of the relay protein in the cells lining the colon is recognized as a factor that promotes tumor growth. The identification and early detection of potential markers of oxidative damage to proteins found in the states that could lead to cancer development (construction of disturbed intestinal crypts, hyperplasia, dysplasia) were considered by Drew’s and his team as one of the elements that would reduce mortality in cancer patients colon.

Although these studies relate to animals, require complicated measurements and indications in the future may translate into practical methods used in the diagnosis of colorectal cancer in humans.

Besides reports on the application marks carbonyl groups in the diagnosis of colorectal cancer advocates to use this parameter in assessing the effectiveness of chemoprevention of tumors with substances of plant origin (20). Chung et al studied the impact of proantocianid compounds on colon cancer cultures cells. As an evaluation parameter oxidative damage to proteins, they used to indicate the carbonyl groups in cell culture homogenate HT-29. The amount of oxidative damage to proteins correlated with changes in content of reactive oxygen species in culture under the influence of antioxidant test.

Demonstrated on the basis of our study, correlating with the clinical stage of the disease, the growth parameters of oxidative damage to proteins in patients with distal colon cancer, suggests the participation of reactive oxygen species in the pathogenesis of this disease. Our results provide the theoretical basis for the use of antioxidant compounds in the chemoprevention of colon cancer.

REFERENCES

1. Korniluk J, Wiślo G, Nurzynski P i wsp.: Epi-
5. Ponczek MB, Wachowicz B: Oddziaływanie re-
10. Stadtman ER, Bedett BS: Reactive oxygen-medi-
13. Popadiuk S, Landowski P, Korzon M i wsp.: Grupy karbonylowe w białkach osocza u dzieci z przewlekłym nieswoistym zapaleniem jelit. Pe-
14. Levine RL: Carbonyl modified proteins in cel-
15. Popadiuk S, Korzon M, Renke J i wsp.: Ocena aktywności reakcji wolnorodnikowych na podstawie analizy peroksydacji białek w powiązaniu z całko-
witą obroną przeciwwutleniaczową krwi u dzieci z chorobą nowotworową, Wiad Lek 1998; 51 Suppl. 4: 107-12.
20. Gawel S, Wardas M, Niedworok E i wsp.: Dialde-