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IMPACT OF CELECOXIB ON SOLUBLE INTERCELLULAR ADHESION MOLECULE-1 AND SOLUBLE E-CADHERIN CONCENTRATIONS IN HUMAN COLON CANCER CELL LINE CULTURES EXPOSED TO PHYTIC ACID AND TNF-α

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Abstract: Soluble adhesion molecules such as soluble intercellular adhesion molecules-1 (sICAM-1) and soluble E-cadherin (sE-cadherin) play important role in tumor invasion and the development of metastasis. It was observed that their concentrations in body fluids of patients with colon cancer were elevated. Celecoxib, a selective inhibitor of cyclooxygenase-2 (COX-2) besides its analgesic, anti-inflammatory, and antipyretic activity is able to inhibit development of colon cancer and reduce risk of metastasis. The additional factors, e.g., dietary components in colon cancer, may influence therapeutic effect of drugs, such as cytokines. TNF-α (tumor necrosis factor – alpha) is a cytokine, which concentration significantly increases in serum of patients with inflammatory and cancer diseases. The latest studies demonstrate, that phytic acid (IP6), a myo-inositol derivative, abundantly present in high-fiber diets could substantially reduce colon cancer incidence. The aim of the present study was to evaluate the influence of celecoxib on sICAM-1 and sE-cadherin concentrations in transformed epithelial colon cell cultures simultaneously exposed to IP6 and TNF-α. Additionally, the adhesion of the exposed cells to collagen I was assessed. HT-29 and Caco-2 cells were cultured in the presence of 50 ng/mL celecoxib, 1.0 mM IP6, and 100 ng/mL TNF-α , and their combination: TNF-α plus IP6, TNF-α plus celecoxib, IP6 plus celecoxib, and TNF-α with celecoxib plus IP6, for 96 h. Nonexposed cell line cultures served as controls. Concentrations of sICAM-1 and sE-cadherin were measured in the culture medium by enzyme-linked immunosorbent assay (ELISA) using Quantikine – Human sICAM-1/CD54 Immunoassay and Quantikine-Human sE-Cadherin Immunoassay. All the results obtained were expressed as ng per mL. In the adhesion assay, the cells were incubated with IP6 (0.5, 1.0 and 2.0 mM), TNF-α (100 ng/mL), celecoxib (50 ng/mL) and their combination for 90 min. Fluorescence values 480 nm/530 nm reflected concentrations of DNA in cells attached to collagen I. The obtained results indicate that celecoxib (50 ng/mL), the selective COX-2 inhibitor, reduces significantly sICAM-1 and sE-cadherin concentrations in HT-29 and Caco-2 transformed human epithelial colorectal cell line cultures co-treated with IP6 (1.0 mM) and TNF-α (100 ng/mL). A decrease of cells adhesion property to collagen I was observed under the influence of 50 ng/mL celecoxib on cell cultures exposed to 1.0 or 2.0 mM IP6 and 1.0 or 2.0 mM IP6 plus 100 ng/mL TNF-α.

Keywords: sICAM-1, sE-cadherin, adhesion, collagen I, celecoxib, phytic acid, TNF-α, HT-29, Caco-2

Information about discovery of antineoplastic activity of non-oncological drugs spreads very quickly. One of them seems to be a class of non-steroidal anti-inflammatory drugs (NSAIDs), coxibs. Celecoxib, a NSAIDs representant, is commonly used to reduce the symptoms and signs of rheumatoid arthritis (RA), osteoarthritis (OA) and ankylosis spondylitis (AS). Celecoxib selectively inhibits cyclooxygenase-2 (COX-2), an enzyme that activates synthesis of prostaglandins, pro-inflammatory molecules, that promote inflammation. Analysis of COX-2 gene expression in colonic epithelium showed that its level was significantly elevated in colon cancer and familial adenomatous polyposis. The phenomenon was not observed in normal colon epithelium. Studies on COX-2 also

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revealed that it is activated in response to different growth factors (VEGF and EGF), cancer promoters (v-src) and cytokines (TNF-α). There are scientific reports that celecoxib, besides its analgesic, anti-inflammatory and antipyretic activity, is able to inhibit development of colon cancer and reduce risk of metastasis (1–5).

It is well known that adhesion molecules such as intercellular adhesion molecules-1 (ICAM-1; CD54) and E-cadherin are involved in carcinogenesis and more often their soluble forms are considered to be a novel promising preclinical cancer serum markers. The measurement of their concentrations in patient's body fluids may be helpful for diagnostics, advancement evaluation, and therapy response monitoring in patients with different types of cancer including colon cancer. (6, 7).

Intercellular adhesion molecule-1 (ICAM-1; CD54) is associated with the processes of cell migration. The membrane ICAM-1 (mICAM-1) is expressed on endothelial and epithelial cells, keratinocytes, dendritic cells, hematopoietic stem cells, hepatocytes, fibroblasts, neutrophils, basophils, eosinophils, lymphocytes, macrophages and monocytes. The most important ligands for ICAM-1 are the B2 integrins LFA-1 (CD1a/CD18) and Mac-1 (CD11b/CD18), which are expressed on leukocytes. As a result of shedding of the molecules ICAM-1 from cells surface into body fluids, they appear in the soluble ICAM-1 (sICAM-1) form. Serum levels of sICAM-1 are elevated in a number of pathology conditions associated with angiogenesis. Furthermore, sICAM-1 may enhance tumor growth by promoting angiogenesis and escape from immunosurveillance. Previous studies have demonstrated that patients with colorectal cancer showed significantly higher levels of sICAM-1 in relation to the control groups (6, 8).

The epithelial transmembrane molecule E-cadherin is the prime mediator of epithelial Ca^{2+}-dependent cell-cell adhesion. It participates in the development and architecture maintenance of epithelial tissues as well as in the signaling process. E-cadherins are dysregulated in the intestinal epithelium in both inflammatory bowel disease and in cancer. It was found that high concentrations of soluble E-cadherin (sE-cadherin) in serum of patients with lung, gastric, prostate and hepatic cancer were positively correlated with a significant advancement of disease and poor survival. sE-cadherin fragments may retain specific biologic activities in the tumor environment favoring aggressiveness and metastasis by antagonizing the intact molecule or serving as an anchor for migration when bound to extracellular matrix (6, 9, 10).

The additional factors, e.g., dietary components in colon cancer and cytokines secreted in the environment of cancer cells may influence therapeutic effect of drugs.

TNF-α (tumor necrosis factor – alpha) is a cytokine, which concentration significantly increases in serum of patients with inflammatory and cancer diseases. The cytokine may increase expression of adhesive molecules (11, 12).

The latest studies demonstrate that phytic acid (IP6), a myo-inositol derivative abundantly present in high-fiber diets, could substantially reduce colon cancer incidence (13). In studies conducted on rat F344 model, it was found that IP6 inhibits pre- and post-initiation stages of colon cancer (14). The latest studies revealed the inhibiting influence of IP6 on adhesion and migration of breast cancer MDA-MB231 cells (15).

The aim of the present study was to evaluate the influence of celecoxib on sICAM-1 and sE-cadherin soluble adhesive molecules concentrations in the colon cancer microenvironment exposed to proinflammatory cytokine TNF-α and to a common food component – phytic acid. Furthermore, adhesive property of colon cancer cells exposed to the listed chemicals to collagen I, one of the extracellular matrix components (16), was estimated.

EXPERIMENTAL

HT-29 and Caco-2 cells derived from human colon *adenocarcinoma* were included into the studies. The cell line HT-29 was kindly provided by Prof. S. Szala (Maria Skłodowska-Curie Memorial Institute of Oncology, Gliwice, Poland). The cell line Caco-2 was purchased from German Collection of Microorganisms and Cell Cultures, Dept. Human Animal Cell Cultures (Braunschweig, Germany).

The cells were cultured under standard conditions in RPMI 1640 medium (Sigma) supplemented with 10% fetal bovine serum (FBS, GIBCO BRL) and antibiotics: 100 U/mL penicillin (Sigma), and 100 μg/mL streptomycin (Sigma) with Nuaire NU-4750 laboratory incubator at stable temperature (37°C), in a humidity of 95% and 5% CO_2. Before the appropriate experiment, the HT-29 and Caco-2 cells were preincubated in 25 cm^2 polystyrene flasks equipped with bacteriological filters (Nunc EasyFlasks™ Nunclon™Δ, Nalge Nunc International). Then, the cells were re-planted with use of 0.25% trypsin (GIBCO BRL) and 1 mM EDTA × 4Na (Life Technologies). Celecoxib was purchased from Sigma-Aldrich. The drug was diluted in DMSO to prepare stock solution. Final drug

concentration (50 ng/mL) was obtained after dilution in RPMI 1640 medium containing antibiotics, 10% serum and 10 mM HEPES buffer (Sigma). In order to expose the cells to phytic acid (IP6), its sodium salt isolated from corn (Sigma) was purchased. After dilution of IP6 with apyrogenic bi-distilled water and stabilization at a pH of 7.4, the solution was filtered through a bacterial filter.

From the IP6 stock solution, final concentrations of 0.5, 1.0 and 2.0 mM were prepared in RPMI 1640 medium containing antibiotics, 10% serum and 10 mM HEPES buffer.

Recombined human TNF-α from Peprotech was processed following the manufacturer's instruction. The reagent in an original vial was centrifuged, reconstituted in apyrogenic bi-distilled water and left in room temperature for 2 h. The stock solution was distributed to sterile tubes and frozen at –20°C. The solution may be stored frozen for up to 3 months. Before the experiment, TNF-α stock solution was thawed and diluted in RPMI 1640 medium containing antibiotics, 10% serum and 10 mM HEPES buffer. TNF-α was used at a final concentration of 100 ng/mL.

In order to estimate sICAM-1 and sE-cadherin concentrations in HT-29 and Caco-2 cells environment, 6×10^4 cells/1.9 cm^2 were seeded in a 24-well culture plate (Nunc). The cells were incubated in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin and streptomycin for 96 h. After 4 days of incubation, a similar culture medium was introduced, additionally supplemented with 10 mM HEPES buffer and tested chemicals: 50 ng/mL celecoxib, 1.0 mM IP6 and 100 ng/mL TNF-α or TNF-α plus IP6, or TNF-α plus celecoxib, or IP6 plus celecoxib, or TNF-α plus celecoxib plus IP6. Non-exposed cell cultures as well as culture medium without cells served as controls. All the cultures listed were exposed to the tested chemicals for the next 96 h. Then, culture medium was collected, centrifuged and frozen at –20°C. sICAM-1 and sE-cadherin concentrations were measured in the culture medium by immunoenzymatic method ELISA using Quantikine – Human sICAM-1/CD54 Immunoassay (R&D Systems) and Quantikine–Human sE-Cadherin Immunoassay (R&D Systems). All the results obtained were expressed as ng per mL based on calibration curves generated with use of analyzed adhesive molecules standards provided by manufacturer with the Quantikine kits. Absorbance was measured at the wavelength of 450 nm with microplate reader MRX Revelation (Dynex, DYNEX™ Software 4.25).

Adhesive properties of HT-29 and Caco-2 cells non-exposed and exposed to celecoxib, IP6 and TNF-α were estimated in 96-well clear collagen I-coated plates (Nunc). A collagen I-coated plate was left at room temperature for 10 min., then blocked with RPMI 1640 medium containing 0.1% serum (30 min.) and washed with RPMI-1640 medium. HT-29 and Caco-2 cells were counted and cells derived from different cultures were bring to the density of 3×10^4 per 200 µL of RPMI 1640 medium containing antibiotics, 0.1% serum, 10 mM HEPES buffer and the tested chemicals: IP6 (0.5, 1.0 and 2.0 mM), TNF-α (100 ng/mL), celecoxib (50 ng/mL), IP6 plus TNF-α, IP6 plus celecoxib, TNF-α plus celecoxib or IP6 plus celecoxib plus

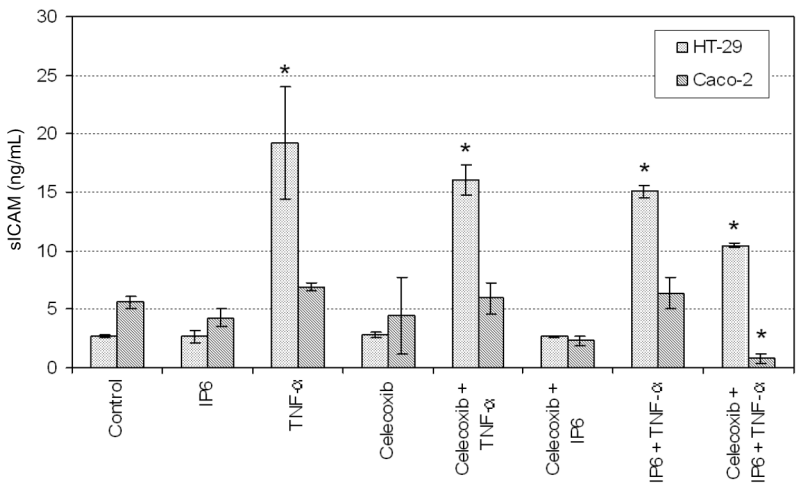

Figure. 1. Effect of 1.0 mM IP6, 100 ng/mL TNF-α and celecoxib on sICAM-1 concentrations in HT-29 and Caco-2 cell cultures. Results are the mean ± SD of 3 experiments. * $p < 0.05$, when compared to the controls

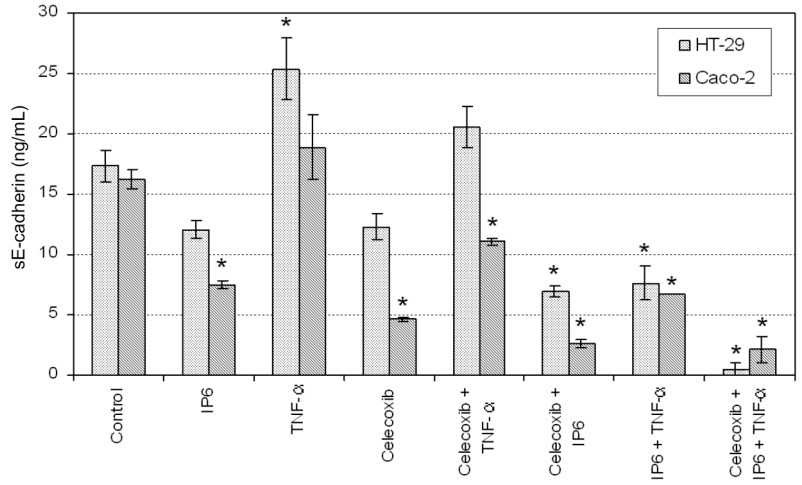

Figure 2. Effect of 1.0 mM IP6, 100 ng/mL TNF-α and 50 ng/mL celecoxib on sE-cadherin concentrations in HT-29 and Caco-2 cell cultures. Results are the mean ± SD of 3 experiments. * $p < 0.05$, when compared to the controls

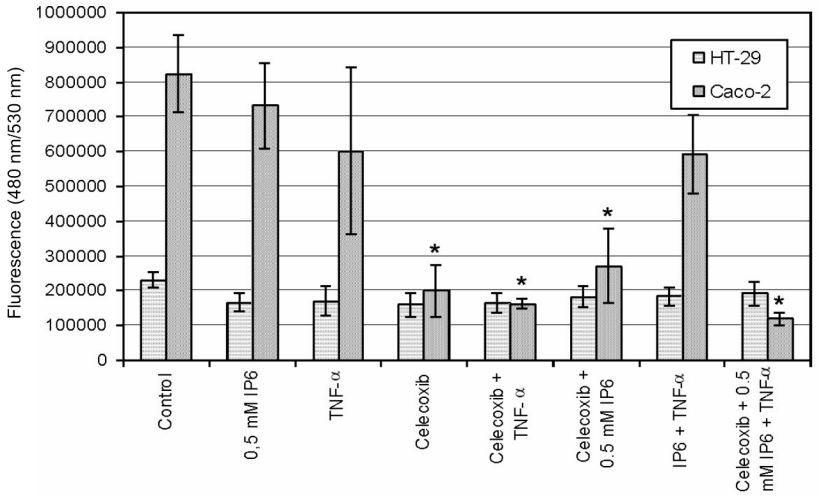

Figure 3. Effect of 0.5 mM IP6, 100 ng/mL TNF-α, and 50 ng/mL celecoxib on HT-29 and Caco-2 cells adhesion to collagen I. Results are the mean ± SD of 3 experiments. * $p < 0.05$, when compared to the controls

TNF-α. The cells were immediately plated into a collagen I-coated plate. Non-exposed cell cultures as well as a culture medium without colon cancer cells served as controls. Following 90 min incubation, culture medium was removed, and a plate was washed four times with 250 µL PBS per well. Quantitative analysis of adhesion property was carried out with the use of Quantikine-Proliferation assay Kit (Cyquant). Next, 200 µL of 1X lysis buffer (Cyquant GR stain, R&D Systems) was added to each well. The stain was diluted 1:300 in the lysis buffer. The plate was shaking at a room temperature for 20 min. Afterwards, 150 µL was transferred from each well to a microplate fitting to the fluorescence reader. The fluorescence was measured at 480530 nm using Victor Light Luminescence counter 1420 TM (Perkin Elmer).

Statistical analysis of sICAM and sE-cadherin concentrations and fluorescence values, which reflect DNA concentration of cells attached to the

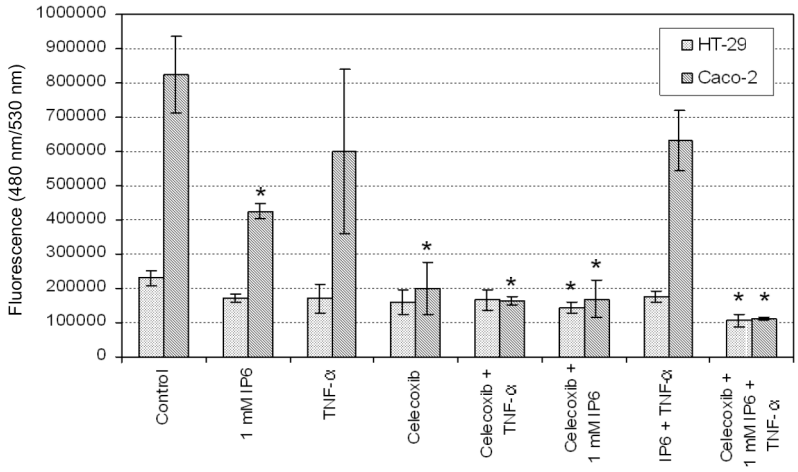

Figure 4. Effect of 1.0 mM IP6, 100 ng/mL TNF-α, and 50 ng/mL celecoxib on HT-29 and Caco-2 cells adhesion to collagen I. Results are the mean ± SD of 3 experiments. * $p < 0.05$, when compared to the controls

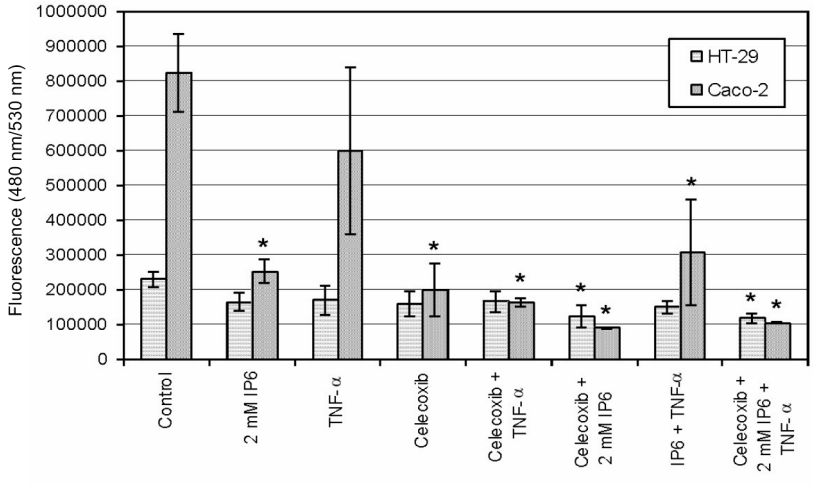

Figure 5. Effect of 2.0 mM IP6, 100 ng/mL TNF-α, and 50 ng/mL celecoxib on HT-29 and Caco-2 cells adhesion to collagen I. Results are the mean ± SD of 3 experiments. * $p < 0.05$, when compared to the controls

substrate, was performed using a model for repeated results (three independent experiments were performed), with arithmetic mean, standard deviation and one-way ANOVA. When the ANOVA results were statistically significant, a single-step multiple comparison procedure (Tukey's test) was used. The statistical analyses were carried out at the $\alpha = 0.05$ significance level, using Statistica PL version 10 software (StatSoft)

RESULTS

The results of our studies showed that mean sICAM-1 concentration in HT-29 cell line control culture was 2.26 ng/mL (± 0.13), while in the control Caco-2 cell line culture amounted 5.56 ng/mL (± 0.58) (Figure 1). Celecoxib (50 ng/mL) alone as well as in the presence of 1.0 mM IP6 or TNF-α did not cause a significant change in this adhesion mol-

ecule concentrations in Caco-2 and HT-29 cell lines either. One mM IP6 alone also did not change concentrations of sICAM-1 in Caco-2 and HT-29 cultures. In both cell line types exposed to a combination of celecoxib, IP6 and TNF-α a statistically significant decrease in sICAM-1 level was observed compared with the cell cultures exposed to a combination of IP6 plus TNF-α (HT-29 p = 0.008; Caco-2 p = 0.008). In HT-29 cell line culture sICAM-1 concentration was statistically significantly higher (p = 0.0002), than in the control culture because of high concentration of the molecule derived from TNF-α exposed cells, but the effect was not observed in Caco-2 cell line cultures.

As it is shown in Figure 2, mean sE-cadherin concentrations in HT-29 and Caco-2 cell line cultures were 17.34 ng/mL (± 1.2) and 16.23 ng/ml (± 0.78), respectively. There was no significant difference in sE-cadherin concentration in HT-29 cell line culture exposed to celecoxib compared with the control one, but in Caco-2 cell line culture it was significantly lower (p = 0.0002). The addition of celecoxib to the HT-29 cell line culture containing TNF-α did not cause changes in sE-cadherin concentration compared with cell culture containing the cytokine exclusively, while in Caco-2 cell line culture it was significantly lower (p = 0.0004) than in culture containing only the cytokine. In both, HT-29 and Caco-2 cell lines, low sE-cadherin concentrations were observed in the presence of celecoxib introduced along with 1 mM IP6 (HT-29 p = 0.0004; Caco-2 p = 0.0002). Co-treatment with celecoxib, IP6 and TNF-α caused a significant decrease in sE-cadherin concentrations in culture medium derived from HT-29 and Caco-2 cell lines down to 0.5 ng/mL (± 0.4) and 2.1 ng/mL (± 1.1), respectively. In HT-29 cell cultures exposed to 1.0 mM IP6 sE-cadherin concentration did not change, but in Caco-2 cells it was significantly lower (p = 0.0002). Additionally, under influence of TNF-α sE-cadherin concentration in HT-29 cell cultures significantly grew (p = 0.02), while in Caco-2 cell cultures it did not change compared with control culture. The co-treatment with IP6 plus TNF-α effect was similar in both cell types. Nevertheless, sE-cadherin concentration in these cultures was significantly lower compared with cultures containing TNF-α (p = 0.0001), but it did not change in comparison to cultures containing only IP6.

Results of our studies on changes in HT-29 cell adhesion under the influence of celecoxib (50 ng/mL), IP6 (0.5, 1.0, 2.0 mM) and TNF-α (100 ng/mL) are shown in Figures 3–5. No changes were seen in adhesion properties of HT-29 cells to collagen I after exposition of the cells to 0.5, 1.0, 2.0 mM IP6, and 100 ng/mL TNF-α in comparison to control culture. Addition of 100 ng/mL TNF-α to the cell cultures incubated with 0.5, 1.0, 2.0 mM IP6 also did not change the number of cells attached to the used base compared with cell culture containing only phytic acid.

The COX-2 inhibitor celecoxib at a concentration of 50 ng/mL also did not cause changes in adhesion of HT-29 cells to collagen I. Otherwise, no changes were observed in the adhesive properties of the cells exposed to 100 ng/mL TNF-α. The reduction of cells adhesion to collagen I (p = 0.008; p = 0.01) was observed only after addition of this drug to the cell cultures containing 1.0 and 2.0 mM IP6. Cells exposed to 1.0 or 2.0 mM IP6 along with TNF-α (100 ng/mL), and celecoxib demonstrated the smallest adhesion to collagen I (p = 0.001; p = 0.005).

Adhesive properties of Caco-2 cells also decreased in the presence of celecoxib (p = 0.0002). A decrease in cell adhesion to collagen I was also observed after addition of the drug to cell cultures exposed to TNF-α (p = 0.007) and 0.5 mM IP6 (p = 0.001). In the presence of 1.0 mM or 2.0 mM IP6 along with celecoxib cell adhesion was significantly lower compared with the control culture (1.0 mM IP6 p = 0001; 2.0 mM IP6 p = 0.0001). It should be pointed out that 1.0 and 2.0 mM IP6 significantly reduced cell adhesion to collagen I (1.0 mM IP6 p = 0.005; 2.0 mM IP6 p = 0.0004). The lowest number of cells attached to the substrate was observed in Caco-2 cell cultures exposed to celecoxib plus IP6, plus TNF-α.

DISCUSSION AND CONCLUSIONS

Colorectal cancer is an extremely invasive type of tumor with aggresive behavior and high metastatic potential (16). A significant number of studies are performed to understand the disease pathogenesis and attempts are making to introduce new therapeutics to complete current therapies. The cyclooxygenase inhibitors (COX-inhibitors) belong to a group of unspecific pharmaceuticals applied for years as anti-inflammatory, antithrombotic, analgetic and antipyretic drugs. However, studies started in the seventies showed a relevant effectiveness of the medicines in epithelium-derived cancer prevention. The most of data were received on colon cancer and celecoxib. Celcoxib, a selective cyclooxygenase-2 (COX-2) inhibitor was approved by American Food and Drug Administration (FDA) for preventive use in patients with diagnosed familial

adenomatous polyposis. The COX-2 isoenzyme is induced by cytokines, growth factors and tumor promoters. Its expression is up-regulated at sites of inflammation and in a variety of malignancies including colon, gastric, esophageal, prostate, pancreatic, breast and lung carcinomas. Studies indicate that overexpression of COX-2 is associated with angiogenesis, decreased host immunity, enhanced invasion, metastasis and poor prognosis. Nonsteroidal anti-inflammatory drugs such as celecoxib mediate anticancer effect by modulating cyclooxygenase-2 (COX-2)-dependent and COX-2-independent mechanisms (1–5). Studies on mechanisms of celecoxib activity still are going on. There are scientific reports that celecoxib administered at low doses in combination with exisulind can prevent prostate cancer (3). There are also scientific articles on non-small cell lung cancer treatment with celecoxib and erlotinib (2).

Herein, we investigated celecoxib in combination with phytic acid (IP6). IP6 is a naturally occuring myo-inositol derivative, abundantly present in high-fiber diets, that can substantially reduce colon cancer incidence. In mammals amount of phytic acid is significantly lower (up to 1.0 mM together with its less phosphorylated derivatives). The latest data confirm the anti-metastatic and anti-angiogenic efficacy of this substance (17–19).

TNF-α is a pro-inflammatory cytokine. It belongs to the group of immunomodulatory cytokines. Some kinds of cancers, including colon cancer have the ability to express TNF-α. It was showed, that in chronic inflammatory and cancer diseases TNF-α overexpression is associated with weight loss and enhanced metabolism driving to cachexia (11, 12).

Numerous studies have indicated that NF-κB activation can suppress cell death pathways and that NF-κB activation is required to protect cells from the apoptotic cascade induced by TNF-α and other stimuli. It was demonstrated that IP6 has an influence on the level of TNF-α gene expression, as well as on the level of expression of genes coding for two TNF-α receptors. The enhanced TNFRI and decreased TNF-α and TNFRII transcription in Caco-2 cells stimulated with IP6 seems to be the presumptive evidence for anti-inflammatory and antitumor activity of IP6 (20).

Taking into consideration the listed mechanism of the drug, which is the diet component and also the cytokine, TNF-α action, we investigated the influence of celecoxib on release of sICAM-1 and sE-cadherins from cancer cells derived from Caco-2 and HT-29 colorectal cancer cell lines exposed to IP6 and TNF-α, and assessed adhesive properties of the cells to collagen I.

Molecules such as ICAM -1 and E-cadherin play a key roles in the invasion and metastasis processes (6, 7). Intercellular adhesion molecules-1 (ICAM-1) belong to immunoglobulin superfamily. Patient whose primary tumors express abundant ICAM-1 in colon have an increased risk of metastasis. It is therefore tempting to speculate whether the expression of ICAM-1 on colonic and pancreatic carcinoma cells may contribute to the aggressive potential of colonic cancer. As a result of shedding of the ICAM-1 molecules from a cell surface into body fluids, they appear in the soluble form of ICAM-1 (sICAM-1). The increase in their concentrations can be both an exponent of the inflammatory process or ongoing neoplastic process (6–8).

Our work indicated that 50 ng/nL celecoxib did not influence the sICAM-1 concentration in both, Caco-2 and HT-29 cell cultures. Celecoxib also neither change the sICAM-1 level in the both cell cultures exposed to IP6 nor in these cultures incubated with TNF-α. Not till then it caused considerable decrease of the molecules concentrations in both types of cell cultures containing TNF-α plus IP6. The sICAM-1 concentrations in cell cultures containing IP6 plus TNF-α and IP6 plus TNF-α and celecoxib were higher than in the control culture. It was due to the high sICAM-1 concentration in HT-29 cell cultures containing 100 ng/mL TNF-α.

In a recent study patients with gastric cancer demonstrated increased levels of sE-cadherin when compared with normal control subjects, and treatment of patients with resectable gastric carcinoma with celecoxib resulted in decreased levels of sE-cadherin. Celecoxib intervention was associated with increased apoptosis and inhibition of angiogenesis. It is known that expression of E-cadherin in the process of invasion and metastasis of gastric carcinoma is regulated by the COX-2. Additionally, it was proven that inhibition of agents that interfere with activation of NF-κB significantly attenuated anomia-induced down-regulation of E-cadherin expression. (6, 9, 10).

Celecoxib caused a decrease of sE-cadherin level in Caco-2 cell cultures and a significant decrease of its level in the cell cultures exposed to TNF-α. When the drug was added to cells incubated with 1.0 mM IP6, the sE-cadherin levels were considerably lower in HT-29 and Caco-2 cultures. On the contrary, the lowest sE-cadherin level was detected in cell cultures containing TNF-α plus IP6, plus celecoxib. It was proved that COX inhibitors induce cancer cells apoptosis through their sensiti-

zation with TNF-α (21, 22). Studies on Caco-2 cells confirmed the reduction of sE-cadherin level compared with cultures containing the cytokine only. However, the lowest sE-cadherin concentration values were noted in cultures containing celecoxib plus IP6, plus TNF-α .

It was demonstrated that the most universally present compound of connective tissue, collagen, can be a chemoattractant inducing the tumor cells invasion (16, 21). Data on colon cancer cells adhesion and concentrations of sICAM and sE-cadherin are very scanty in the literature. In the most cases of research each chemical is tested individually. Tantivejkul et al. (15) showed that in breast cancer MDA-MB 231 cells 2 mM phytic acid caused 65% inhibition in the cells attachment to fibronectin and 37% inhibition of attachment to collagen. There are opinions that TNF-α can contribute to the metastasis formation.

Our results demonstrated that adhesion to collagen I decreased in the both cell lines in the presence of celecoxib plus 1.0 or 2.0 mM IP6 and 1.0 or 2.0 mM IP6 plus TNF-α plus celecoxib. In Caco-2 cell line the decrease of adhesion to collagen I was observed in the presence of celecoxib and 1.0 or 2.0 mM IP6. In the adhesion test the cells were exposed to the tested chemicals only for 90 min. Although, the differences in the cells adhesion were observed. Cells were suspended in the tested solutions after trypsinization. Therefore, the number of cells attached to collagen may suggest the ability of these cells to metastasize.

The obtained results indicate that celecoxib (50 ng/mL), the selective COX-2 inhibitor, reduces significantly sICAM-1 and sE-cadherin concentrations in HT-29 and Caco-2 transformed human epithelial colorectal cell line cultures co-treated with phytic acid (1.0 mM) and TNF-α (100 ng/mL). A decrease of cancer cells adhesion to collagen I was observed under the influence of 50 ng/mL celecoxib on cell cultures exposed to 1.0 or 2.0 mM IP6 and 1.0 or 2.0 mM IP6 plus 100 ng/mL TNF-α.

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