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The activity of serum beta-galactosidase in colon cancer patients with a history of alcohol and nicotine dependence: preliminary data

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Introduction: Beta-galactosidase (GAL) is a lysosomal exoglycosidase involved in the catabolism of glycoconjugates through the sequential release of beta-linked terminal galactosyl residues. The stimulation of activity of exoglycosidases and other degradative enzymes has been noted in cancers as well as in alcohol and nicotine addiction separately. This is the first study to evaluate the activity of the serum senescence marker GAL in colon cancer patients with a history of alcohol and nicotine dependence, as a potential factor of worse cancer prognosis.

Material and Methods: The material was serum of 18 colon cancer patients and 10 healthy volunteers. Ten colon cancer patients met alcohol and nicotine dependence criteria. The activity of beta-galactosidase (pkat/ml) was determined by the colorimetric method. Comparisons between groups were made using the Kruskal-Wallis analysis and differences evaluated using the Mann-Whitney U test. Spearman's rank correlation coefficient was used to measure the statistical dependence between two variables.

Results: The activity of serum GAL was significantly higher in colon cancer patients with a history of alcohol and nicotine dependence, in comparison to colon cancer patients without a history of drinking/smoking (p=0.015; 46% increase), and the controls (p=0.0002; 81% increase). The activity of serum GAL in colon cancer patients without a history of alcohol/nicotine dependence was higher than the activity in the controls (p = 0.043; 24% increase).

Discussion/ Conclusion: Higher activity of beta-galactosidase may potentially reflect the accelerated growth of the cancer, invasion, metastases, and maturation, when alcohol and nicotine dependence coincide with colon cancer. For a better prognosis of colon cancer, alcohol and nicotine withdrawal seems to be required.

Keywords: **β-galactosidase • alcohol • smoking • colon cancer**

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Abbreviations: **ADH1C*1** – alcohol dehydrogenase gene; **ALDH2*2** – aldehyde dehydrogenase gene; **FAEEs** – fatty acid ethyl esters; **FUC** – α-fucosidase; **GAL** – β-galactosidase; **HEX** – β-hexosaminidase; **P-450 2E1** – cytochrome; **ROS** – reactive oxygen species

Introduction

Colon and rectal cancers accounted for more than 9% of total worldwide cancer cases [6]. Epidemiological studies found more than 7-fold higher risk for distal colorectal cancer in individuals who consume more than 20 g of ethanol a day and have subsequent low methionine and folate levels, as compared to occasional drinkers. The following risk factors for alcohol-associated colon carcinogenesis exist: chronic inflammatory bowel disease, polyps, folate deficiency, alcohol dehydrogenase gene ADH1C*1 homozygosity and aldehyde dehydrogenase gene ALDH2*2 mutations (that increase acetaldehyde concentrations) [11]. It is also known that alcohol and tobacco are the most abundantly consumed noxious compounds worldwide that act synergistically, resulting in an increased cancer risk [14,20]. Approximately 80% of alcoholics smoke cigarettes [20]. Acetaldehyde found in alcoholics who smoke comes from ingested ethanol and tobacco smoke. Acetaldehyde is known to be present in a high concentration in the large intestine after alcohol consumption, corresponding to the blood level [3,11]. Besides acetaldehyde, tobacco smoke is a source of oxidative stress, and contains up to 3000 toxic substances, such as nicotine, nitrosamines, carbon monoxide, and other aldehydes, that may damage the tissues. Moreover, reactive oxygen species (ROS) generated during drinking and smoking, as well as non-oxidative metabolites of ethanol (e.g., fatty acid ethyl esters – FAEEs) and the ethanol–water competition mechanism, might be involved in the resulting damage of the alimentary tract tissues [20,23,25]. Acetaldehyde was found to be responsible for carcinogenesis, since it acts directly to cause cellular injury and proliferation, it decreases glutathione levels and ROS elimination, it is carcinogenic, mutagenic, binds to DNA and proteins, destroys folate and results in secondary hyperregeneration [6,11]. Chronic alcohol consumption was found to induce cytochrome P-450 2E1 in gastrointestinal mucosa cells and in the liver, resulting in the increased generation of ROS and increased activation of various dietary and environmental carcinogens such as those present in tobacco smoke and diet (e.g. polycyclic hydrocarbons, hydrazines and nitrosamines) [11]. Increased amounts of the lipid peroxidation products of ROS bind to DNA, forming highly mutagenic adducts [14]. Induced cytochrome P-450 2E1 also decreases tissue levels of retinol and retinoic acid that have important functions in the regulation of cell growth and differentiation action. Usual nutritional deficiencies observed in alcoholics and disturbed methyl transfer result in inadequate DNA synthesis and repair [11,14]. Although alcohol and smoking act synergistically, alcohol abuse is associated with more than three times greater risk of developing cancer than smoking (data for oesophageal carcinoma) [14].

Beta-galactosidase (GAL) is a lysosomal exoglycosidase involved in the catabolism of glycoconjugates by sequential release of beta-linked terminal galactosyl residues. GAL expression has been shown to be a reliable indicator of the switch mechanism used by cells to enter senescence [16,19]. The senescent phenotype displays distinct morphological characteristics where cells become enlarged and flattened with increased granularity and a vacuole-rich cytoplasm [13]. The increased serum activity of beta-galactosidase in invasive colon tumours has been reported earlier [5,17]. It has also been noted that alcohol abuse and smoking increase the activity of some serum exoglycosidases (e.g. β-hexosaminidase) [20]. Malignant tumours produce various hydrolases, including beta-galactosidase, which degrade pericancerous matrix, favouring tumour growth, invasion and metastatic propagation [5,17]. The proteolysis of matrix glycoproteins depends on the initial removal of the carbohydrate side chains. Since the stimulation of activity of glycosidases and other degradative enzymes has been associated with tumour development as well as with alcohol and nicotine addiction, the activity of beta-galactosidase in colon cancer patients with a history of alcohol and smoking dependence may be higher than in patients without such a history.

Therefore, we decided to compare for the first time the activity of serum GAL (a senescence marker) between alcohol- and nicotine-dependent colon cancer patients

and colon cancer patients without a history of alcohol and nicotine dependence.

Materials and methods

The serum of 18 colon cancer patients (10 females and 8 males; mean age 67) and 10 healthy volunteers (6 females and 4 males; mean age 45) was obtained in the Department of General and Endocrinological Surgery of the Medical University of Białystok. Patients suffering from cancer had histopathologically diagnosed colon adenocarcinoma. According to the clinical-pathological classification of TNM and Duke's classification [17], tumours with cell differentiation G2 (n = 16) and G3 (n = 2) were found in patients operated on for colon adenocarcinoma. Patient groups were selected according to the extent of spread of colon adenocarcinoma: pT1 (n = 1), pT2 (n = 7), pT3 (n = 7) and pT4 (n = 3). In the macroscopic examination, groups were selected depending on the diameter of the resected tumour: 1-3.5 cm (n = 11), 4 cm (n = 4) and 5-7 cm (n = 3). Ten colon cancer patients met the ICD-10 and DSM-IV criteria for alcohol and nicotine dependence.

The activity of β-galactosidase in the serum (pkat/ml) was assayed in duplicate by the colorimetric determination of p-nitrophenol released from p-nitrophenyl-β-D-galactopyranoside (Sigma, USA) by β-galactosidase [19]. The mixture of enzyme and substrate was incubated for 60 min at 37°C.

The study was approved by the local Bioethical Committee of the Medical University of Białystok and was conducted in accordance with the Helsinki Declaration. Informed written consent was obtained from all the subjects after explanation of the nature, purpose, and potential risks of the study.

Statistical analysis

Normality was estimated by using a Kolmogorov–Smirnov test. Comparisons between groups were made using Kruskal-Wallis analysis and differences were evaluated using the Mann-Whitney U-test. Statistical analysis was performed with Statistica version 10.0 (StatSoft, Kraków, Poland). Statistical significance was assumed at $p < 0.05$.

Results

As Figure 1 shows, the activity of the serum GAL (pkat/ml) in colon cancer patients dependent on alcohol and nicotine (130 ± 38 pkat/ml) was significantly higher than in the serum of colon cancer patients without a history of drinking/smoking (89 ± 16 pkat/ml) ($p = 0.015$*) and in the control group (72 ± 12 pkat/ml) ($p = 0.0002$***). The activity of serum GAL in colon cancer patients without a history of drinking/smoking was significantly higher than in the control group ($p = 0.043$*). There were no correlations between the serum GAL activity and stages of cell differentiation (G) and of the extent of spread (TNM) of colon adenocarcinoma.

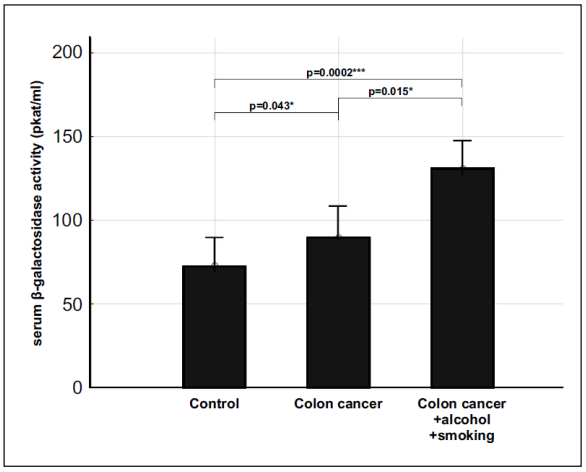

Figure 1. Activity of serum β-galactosidase in the control group, colon cancer patients without a history of alcohol drinking or smoking, and in the colon cancer patients with alcohol and nicotine dependence

Discussion

The tendency to increased salivary GAL activity was noted earlier after a single large acute alcohol intoxication [22], whereas in serum of alcoholic patients no significant changes in GAL activity were found when compared to the controls [15]. It was found that hyposialylated forms of transferrin with terminal galactose residues are less eliminated by the hepatocytes, and become senescent glycoproteins [7]. It was also found that cells in multiple exposures to cigarette smoke extract had profound growth arrest, a flat and enlarged morphology, upregulated retinoblastoma protein pathways (p16), and increased senescence-associated GAL activity [10]. On the other hand, no significant differences in the salivary GAL specific activity were found between smokers and nonsmokers [9].

In our study, we found that patients suffering from colon cancer have higher (24%) serum GAL activity than controls ($p = 0.043$), whereas colon cancer patients dependent on nicotine and alcohol have much higher (81%) GAL activity than controls ($p = 0.0002$) and higher (46%) than colon cancer patients without a history of drinking/smoking ($p = 0.015$; Figure 1). It is widely known that the activity of glycosidases is higher in young tissue [4,18]. Therefore, the activity of lysosomal exoglycosidases in tissues obtained during surgical procedures may be a helpful marker in distinguishing between benign/slowly-growing and malignant tumours, being also crucial in the prognosis and strategy of treatment of neoplastic diseases. The highest activities of exoglycosidases were observed in high-grade tumours, which correlated with the degree of malignancy, suggesting that activity of exoglycosidases is dependent on the phase of tumour development [5].

A significant increase was reported in the activity of β-hexosaminidase (HEX), α-fucosidase (FUC) and

β-galactosidase (GAL), in the serum of patients with colon cancer, when compared to the healthy controls. The determination of the serum activity of HEX, FUC and GAL of patients with colon cancer had a high diagnostic value. In the urine of colon cancer patients, HEX and GAL activity was also increased, with a high diagnostic value. Increased lysosomal exoglycosidases were also described in serum and urine of patients suffering from pancreatic cancer [17], in tissue of brain, lung, stomach, prostate, thyroid, cervical and breast cancer [2,4,8,27]. As glycoconjugates from the cell surface are involved in important cellular and molecular processes such as cell adhesion, growth and proliferation, cell-cell interactions, division, differentiation, and signal transduction [8], exoglycosidases may be crucial in the cell transformation to the primary cancerous, localized tumour growth, and to metastatic propagation. The malignancy process causes an increase in cellular death, releasing lysosomal enzymes from the lysosomes, or causes an increase in the synthesis rate or disturbs the packaging of the lysosomal enzymes in the lysosomes, thus increasing activity of hydrolases in the body fluids [1].

As alcohol abuse is associated with more than three times greater risk of developing cancer than smoking [14], higher activity of GAL in smoking alcoholics suffering from colon cancer than colon cancer patients without a history of drinking/smoking seems mostly to be due to the action of alcohol. Although there is no clear evidence that alcohol drinking or smoking increase GAL activity (only a tendency to the increase was found in the saliva of binge drinkers [22]), we clearly showed that alcoholism and smoking (together) significantly increased GAL activity in the colon cancer patients (41% increase). The lack of correlations between the serum GAL activity and stages of cell differentiation (G) and of the extent of spread (TNM) of colon adenocarcinoma may be due to the superimposed increase in GAL activity induced by the action of alcohol and smoking. As increased exoglycosidases are involved in cancer propagation and metastases [5,17], alcohol drinking and smoking may potentially accelerate tumour growth, invasion and metastasis. Since GAL is known to be associated with the senescence process [19], the metabolites from alcohol drinking and cigarette smoking such as acetaldehyde and ROS as well as decreased immunity caused by the alcohol and cigarettes [12 ,21,22,24,26] might favour not only tumour growth, invasion and metastases, but also maturation of the colon cancer tissue.

Conclusions

Chronic alcohol drinking and cigarette smoking, acting synergistically on the cancer process, by increasing β-galactosidase activity, may potentially worsen the prognosis of colon cancer. Therefore alcohol and nicotine withdrawal seems to be required to improve the prognosis of colon cancer.

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