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Serine-threonine protein kinase activities under colitis-associated carcinogenesis development

Aktywność kinazy białkowej seryna-treonina w przebiegu karcinogenezy związanej z zapaleniem okrężnicy

INTRODUCTION

Nonspecific ulcerative colitis is a chronic inflammatory process with unknown etiology, which is characterized by dystrophic and atrophic destructive changes in colon mucosa. Such disorders are accompanied by secretory and motor functions of colon disturbances [6]. The imbalance of water and electrolytes transport in colon is one of the most important cytotoxic factors of ulcerative colitis development. These processes correlate with fluctuations of such intracellular second messengers as cAMP, cGMP and Ca^{2+}. These signal molecules provide the activation of cAMP-, cGMP- and Ca^{2+}-dependent protein kinases. The kinases are enzymes involved in mechanisms of ion transport, proliferation and differentiation control by phosphorylation of different protein substrates [9]. Experimental investigations have shown that serine-threonine protein kinases (PKA, PKG and PKC) activation result in phosphorylation of Na^+-channels and Na^+/H^+-exchanger and inhibition of Na^+ absorption.

The aim of the study was to research the serine-threonine protein kinase activities in membrane and cytoplasmic fractions of colon mucosa epithelial cell under colitis-associated carcinogenesis development.

MATERIAL AND METHODS

Male white rats with initial weight 160–180 g were used in the experiment. Experimental colitis-associated carcinogenesis development was induced according to method [3]. Rats were given water containing 1% (w/v) dextran sulfate sodium salt (DSS ICN m. w. ≈ 40,000), ad libitum for 7 days for colitis modeling. Then followed weekly subcutaneous injections of procarcinogen 1,2-dimethylhydrazine (DMH; Sigma-Aldrich) 20 mg/kg body weight during 10 weeks. Pathology

development was identified visually and histologicaly in colon mucosa. The rats were sacrificed on first, third and seventh days of DSS treatment and on second, fourth, sixth, eighth and tenth weeks during DMG influence. Their large bowels were rapidly removed and flushed with saline. The large bowels were macroscopically inspected, cut, and fixed in 10% buffered formalin for histopathological examination. Besides, colonocyte was isolated based on a method for a rat colon [7]. The membrane and cytoplasmic fraction of colonocyte were intercepted according to [8]. The colonocyte activity of protein kinase C was determined as previously described [5] by measuring the transfer of the phosphate group from [γ-32P]ATP (GE Healthcare) to the specific substrate histone H1 (Sigma-Aldrich). PKA and PKG activity was assayed by the phosphorylation of a specific substrate kemptide (Sigma-Aldrich), with [γ-32P] ATP as described previously [2]. After incubation, the reactions were stopped by spotting the reaction mixture on Whatman, dried and washed four times with agitation in 10% trichloracetic acid, followed by soaking in acetone and air drying. The dried papers were counted for ^{32}P incorporated radioactivity in 10 ml of scintillation fluid ЖС-107 by scintillation counter "Delta". Protein concentration was measured by Bradford method using bovine serum albumin as a standard [1].

Results are expressed as the mean ± standard error (SE). Student's t test was used to determine statistical significance between control and test groups. A p value of < 0.05 was considered statistically significant.

RESULTS

The investigation of serine-threonine protein-kinase activities in epitheliocyte membrane and cytoplasmic fractions under colitis-associated carcinogenesis development showed intracellular redistribution of enzyme activity (Fig.1).

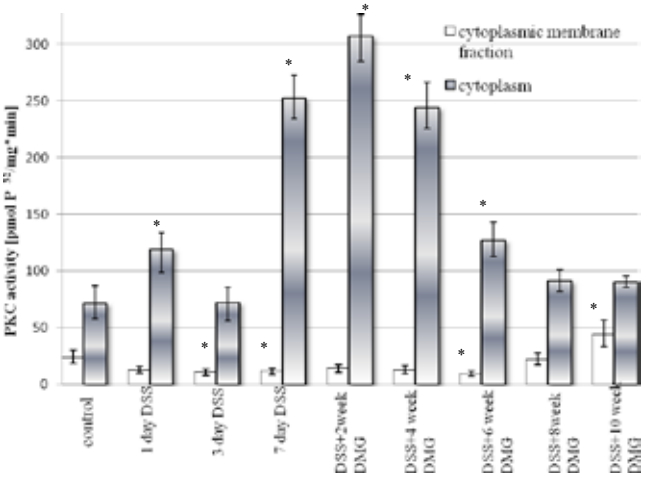

Fig. 1. PKC activity in cytoplasmic membrane and cytoplasm of rat colon epitheliocytes under colitis-associated carcinogenesis; * $P \leq 0.05$

Decrease of PKC activity was established in cytoplasmic membrane fraction on the first day of DSS treatment. The same parameter in cell cytoplasm increased 1.7 times during this period of experiment. The determined changes of PKC activity in the membrane were probably caused by structural and functional disorders of epithelial cell membrane state under inflammation and malignisation. Such disorders in mucous cell membrane were shown by us earlier [4]. Cytoplasmic PKC activity had control value on the third day of the experiment, but on the 7-th day it increased, exceeding the reference value more than 3.5 times. Consequent rising of PKC activity in colon epithelial cell cytoplasm was established under weekly procarcinogen introduction. In particular, observable enzyme activity exceeded the control more than 4 times on the second week of DMG treatment. Obtained data allow us to assume that inflammation processes accompanying ulcerative colitis were triggers for abnormal calcium uptake, PKC activation, which might contribute to intensification of uncontrolled cell proliferation, inhibition of differentiation and apoptosis [10]. The cAMP-dependent protein kinase (PKA) activity showed an increase of the enzyme activity only on the 7-th day of colitis development. The parameter was more than 2 times higher comparing with the control both in membrane and cytoplasmic fractions of colon mucosal cell (Fig. 2).

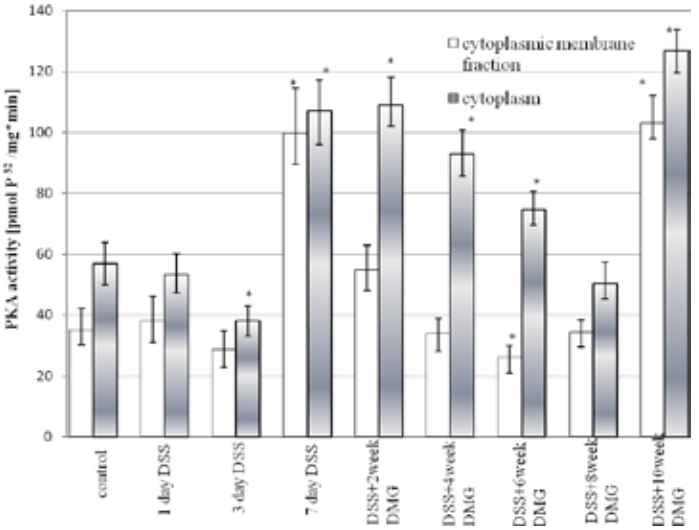

Fig. 2. PKA activity in cytoplasmic membrane and cytoplasm of rat colon epitheliocytes under colitis-associated carcinogenesis; * $P \leq 0.05$

Some redistribution of PKA activity was observed after 2 weekly injections of specific procarcinogen. The investigated enzyme index in cytoplasm remained enhanced and in membrane fraction the PKA activity receded nearly to control value. Gradually, a decrease of PKA activities in cytoplasmic and membrane fractions was established during the next time point of experiment (4 and 6 weeks). cAMP-dependent protein kinase activity reached the reference value on 8 week of DMG influence.

The investigation of cGMP-dependent protein kinase (PKG) activity showed significant reduction of this parameter in membrane (on 1-st day of DSS treatment) and in cytoplasmic (1-st, 3-rd days) fractions of colon mucous cell (Fig. 3).

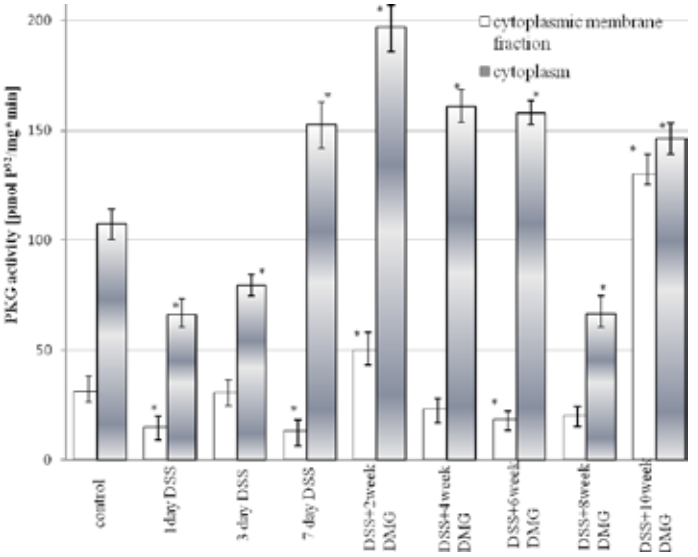

Fig. 3. PKG activity in cytoplasmic membrane and cytoplasm of rat colon epitheliocytes under colitis-associated carcinogenesis; * $P \leq 0.05$

A significant decrease by 50% in regard to control of membrane associated PKG activity was established on 7-th day of colon inflammation stimulation. The activity of cytoplasmic isoforms of the investigated enzyme was half as much as the control at this point of the experiment. Procarcinogen (DMG) administration was accompanied by increasing of cytoplasmic and membrane PKG activity. This parameter exceeded control value almost two times and reached its peak value during the experiment for cytoplasm on the second week of dimethylgidrazine influence. The later terms of carcinogenesis (4, 6 weeks) were characterized by rising, comparing the reference, PKG activity in cytoplasm and reduction of this index in membrane fractions.

The obtained results allowed us to state that subject to pathology process stage both ulcerative colitis development and earlier phases of colon malignisation were conducted with functional disturbances of major enzymes in cyclic nucleotide-dependent signal pathways.

CONCLUSIONS

1. The decrease of membrane and cytoplasmic-asossiated PKG activities and depression of cAMP-, cGMP-dependent protein kinase activities in colon epitheliocyte cytoplasmic fraction were shown during initial phase of inflammation (1–3 days).

2. Later stages of inflammation (7 day) and following malignant transformation of rat colon mucous cell (2–6 weeks) were characterized by serine-threonine protein kinases activities increase in cytoplasmic fraction of the cell.

3. The increase of all investigated protein kinases activity was established in membrane fraction of epiheliocytes under colon adenocarcinoma diagnosed on 10-th week of the experiment.

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SUMMARY

Kinases are enzymes involved in mechanisms of ion transport, proliferation and differentiation control by phosphorylation of different protein substrates. The aim of the study was to research the serine-threonine protein kinase activities in membrane and cytoplasmic fractions of colon mucosa epithelial cell under colitis-associated carcinogenesis development.

Keywords: ulcerative colitis, epitheliocytes, serine-threonine protein kinases, colitis-associated carcinogenesis

STRESZCZENIE

Kinazy są enzymami uczestniczącymi w mechanizmach transportu jonów, kontroli proliferacji i różnicowania komórek poprzez fosforylację różnych substratów białkowych. Celem przeprowadzonych badań była ocena aktywności kinazy białkowej seryna-treonina frakcji błonowej i cytoplazmatycznej komórek nabłonkowych śluzówki okrężnicy w przebiegu karcinogenezy związanej z zapaleniem jelita grubego.

Słowa kluczowe: wrzodziejące zapalenie okrężnicy, epiteliocyty, kinazy białkowe seryna-treonina, karcinogeneza związana z zapaleniem okrężnicy