ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 3, 24 SECTIO DDD 2010

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Effect of maleimide derivative on oxidative stress and glutathione antioxidant system in 1,2-dimethylhydrazine induced colon carcinogenesis in rat

Wpływ pochodnych maleimidu na stres oksydacyjny i glutationowy system antyoksydacyjny w karcinogenezie okrężnicy indukowanej 1,2-dimetylohydrazyną

INTRODUCTION

Protein kinases are the most exploited targets in modern drug discovery due to key the roles of these enzymes in human diseases, including cancer [5]. Novel protein kinases inhibitor maleimide derivative 1-(4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1H-pyrrol-2.5-dione (MI-1) has been synthesized at the Taras Shevchenko National University of Kyiv. MI-1 exhibits cytostatic effects on interferon resistant cell line, transformed cell lines HEK293, *MCF-7;* the most significant influence has been determined on SW620 (human caucasian colon adenocarcinoma) [4]. The 1,2-dimethylhydrazine (DMH)-induced colon cancer is morphologically similar to human colon cancer [8].

The aim was to investigate the levels of protein carbonyl groups and thiobarbituric acid reactive substances (TBARS) and glutathione antioxidant system after treatment with MI-1 in case of DMH-induced colon cancer in rats and c-kit expression that is often mutated in colorectal carcinoma.

MATERIAL AND METHODS

MI-1 as a fat-soluble substance was dissolved in sunflower oil and injected by intragastric way in doses 0.027, and 2.7 mg/kg b.w. everyday for 20 weeks. Colon carcinogenesis was induced by weekly subcutaneous injections of DMH dissolved in physiological solution (20 mg/kg body weight) for 20 weeks. Rats were divided into 8 groups, 3 of them are controls. Group 1 (control) was administered physiological solution, group 2 – DMH, group 3 (control) – sunflower oil, group 4 – MI-1, 0.027 mg/

kg, group 5 – MI-1 2.7 mg/kg, group 6 – physiological solution and sunflower oil, group 7 – DMH and MI-1, 0.027 mg/kg, group 8 – DMH and MI-1, 2.7 mg/kg.

The contents of thiobarbituric acid reactive substances (TBARS) [9], protein carbonyl groups [7], reduced glutathione (GSH), and glutathione peroxidase (GSHPx), glutathione-S-transferase (GST) activities [9] were determined in intestinal mucosa homogenate. The protein level of *c-kit tyrosine kinase (CD117, 145 kDa)* was identified using *Western blotting*.

RESULTS

The content of protein carbonyl groups and TBARS (Fig. 1) in the intestinal mucosa homogenate was significantly increased (nearly twice) than control sample. MI-1 does not change the level of protein carbonyl groups in dose 0.027 mg/kg, while in dose 2.7 mg/kg this parameter is diminished by 30%. But TBARS concentration is decreased by 29% in both doses. Both MI-1 and its combined administration with DMH did not cause significant changes of these parameters.

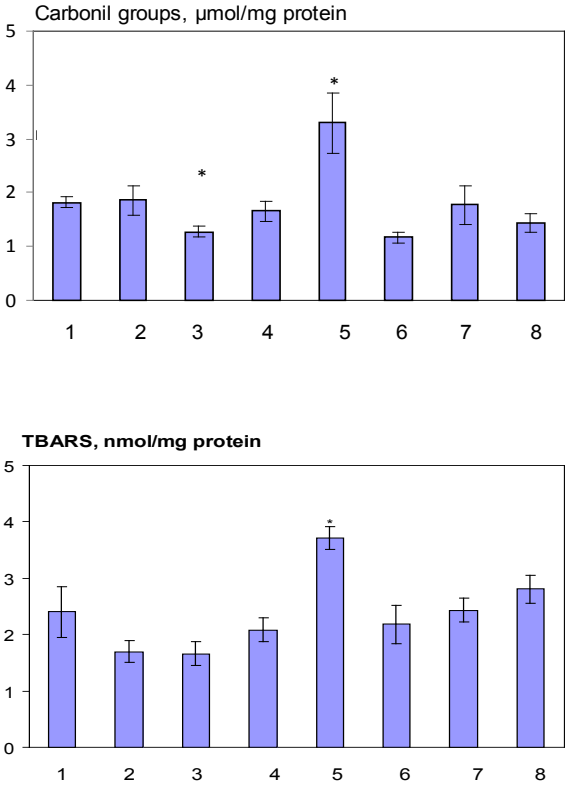

Fig. 1. Content of carbonil groups and TBARS in intestinal mucosa of rats: 1 – control, sunflower oil; 2 – MI-1, 0.027 mg/kg; 3 – MI-1, 2.7 mg/kg; 4 – control, physiological solution; 5 – DMH; 6 – control, physiological solution + sunflower oil; 7 – DMH + MI-1, 0.027 mg/kg; 8 - DMH + MI-1, 2.7 mg/kg

In DMH treated rats the levels of GSH-dependent parameters were elevated in comparison to control rats. DMH significantly increases the level of GSH by 90%, GST activity by 62% and insignificantly GSHPx activity by 26% (Tab. 1). MI-1 reveals a tendency to decrease GSHPx activity by 27% in both doses and insignificantly decreases GST activity and the level of GSH. The co-administration of DMH and MI-1 did not cause significant changes of the explored parameters.

Table 1. The level of reduced glutathione and glutathione peroxidase and glutathione-S-transferase activities in intestinal mucosa of rats

Groups	GSH (nmol/ mg protein · min)	GSHPx (nmol/ mg protein · min)	GST (μmol/ mg protein · min)
Control (oil)	1.03 ± 0.13	4.14 ± 0.09	0.16 ± 0.01
MI-1 0.027 mg/kg	0.89 ± 0.03	3.20 ± 0.25	0.14 ± 0.03
MI-1 2.7 mg/kg	0.97 ± 0.14	3.01 ±0.31*	0.13 ± 0.02
Control (phys. sol.)	0.83 ± 0.09	3.88 ±0.40	0.08 ± 0.01
DMH	1.53 ± 0.30*	4.89 ± 0.49	0.13 ± 0.02
Control (phys.sol.+ oil)	1.04 ± 0.01	3.93 ± 0.49	0.20 ± 0.03
DMH+MI-1 0.027 mg/kg	1.16 ± 0.16	4.44 ± 0.49	0.22 ± 0.03
DMH+MI-1 2.7 mg/kg	1.30 ± 0.11	4.87 ± 0.70	0.15 ± 0.02

Values are Mean ± SD derived from 8 rats. * – $p < 0.05$ compared with control group

The c-kit antibody detected one major band between 130 and 170 kDa which corresponds to Mr 145 kDa (Fig. 2). This band represents the c-kit tyrosine kinase receptor. The protein level of the c-kit was detected only on two tracks corresponding to DMH-treated group and its combined administration with MI-1 in dose 0.027 mg/kg.

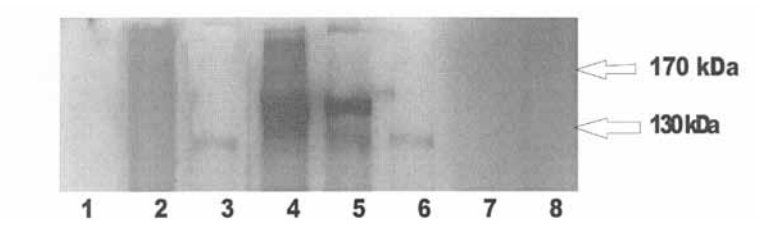

Fig. 2. The protein level of c-kit tyrosine kinase (Mr 145 kDa) in rats; 1 – control, sunflower oil; 2 – control, physiological solution; 3 – control, physiological solution + sunflower oil; 4 – DMH; 5 – DMH + MI-1, 0.027 mg/kg; 6 – MI-1, 0.027 mg/kg; 7 – DMH + MI-1, 2.7 mg/kg; 8 – MI-1, 2.7 mg/kg

DISCUSSION

The increase of the level of TBARS and protein carbonyl groups in DMH-treated rats has been shown. Theses results correlate with our previous finding [6] and increased plasma and tissue malone

dialdehide (MDA) content in colorectal cancer patients [2]. It has been claimed that MDA acts as a tumor promoter and co-carcinogenic agent because of its high cytotoxicity and inhibitory action on protective enzymes. However, MI-1 administration to DMH treated animals showed a reduction in the levels of TBARS. It was attributed to MI-1 antiperoxidative property.

GSH and GSH-dependent enzymes are important in neoplastis diseases and they play a crucial role in the defence against reactive oxygen species, detoxification of xenobiotics and carcinogens [3]. We observed enhanced levels of GSH and GST, GSHPx activities in intestinal mucosa of DMH-treated rats. This may be due to the increased cell proliferation involved in the pathogenesis of DMH-induced colon cancer. It was previously demonstrated that GSH, GSHPx, GST and GSH-reductase was expressed in greater amounts in neoplastic cells, conferring a selective growth advantage [8]. Other studies showed similar changes in GSH-dependent enzymes in cases of colorectal cancer, while GSH level increased or decreased depending on studies [10]. MI-1 caused a tendency to decrease GSHPx activity; however, the level of GSH, as substrate for GSHPx, did not change. On the administration of MI-1 to DMH-treated rats, the GSH levels and GSHPx, GST activities were decreased to control values. It suggests that MI-1 reveals the ability to inhibit the progression of cancer.

The expression of the c-kit proto-oncogene has been reported in a number of cancer cell lines, and human colorectal tumors also express this proto-oncogene [1]. It is known that the activation of the c-Kit/SCF pathway has been detected in several human colon cancer cell lines. We revealed the level of c-kit only in DMH-treated group and its combined administration with MI-1 in dose 0.027 mg/kg, whereas MI-1 in dose 2.7 mg/kg blocks c-kit expression in DMH-induced colon carcinogenesis in rat and might be a potential chemotherapeutic agent for the treatment of colon cancer.

CONCLUSIONS

Novel maleimide derivative MI-1 did not significantly disturb peroxidation/antioxidation system of intestinal mucosa, and caused recovery of the contents of TBARS, protein carbonyl groups, GSH, and GSHPx, GST activities in DMH-treated rats. MI-1 blocks expression of c-kit proto-oncogene in DMH-induced colon carcinogenesis in rat. Therefore, MI-1 might be a potential chemotherapeutic agent for the treatment of colon cancer.

This study was supported by President of Ukraine Grant for Young Scientist (N 336/2008-рп 16.12.2009).

REFERENCES

1. Attoub S. et al.: The c-kit tyrosine kinase inhibitor ST1571 for colorectal cancer therapy. Cancer Res., 62, 4879, 2002.
2. Chadha V. D. et al.: Zinc mediated normalization of histoarchitecture and antioxidant status offers protection against initiation of experimental carcinogenesis. Mol. Cell Biochem., 304, 101, 2007.
3. Czeczot H. et al.: Glutathione and GSH-dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma. Acta Biochim. Polonica, 53, 237, 2006.

4. Dubinina G. G. et al.: Antiproliferative action of the new derivatives of 1-(4-R-benzyl)-3-R1-4-(R2-phenylamino)-1H-pyrrol-2,5-dione. Zh. Org. Farm. Chim., 5, 39, 2007.
5. Dubinina G.G. et al.: *In Silico* design of protein kinase inhibitors: successes and failures. Anti-Cancer Agents in Med. Chem., 7, 171, 2007.
6. Filinska O. et al.: The lipid peroxidation and the antioxidant system of the rat liver during 1,2-dimethylhidrazine-induced carcinogenesis. The Ukr. Biochem. J., 81, 196, 2009.
7. Levine R.L. et al.: Method in enzymology, 186, 464, 1990.
8. Manju V. et al.: Rat colonic lipid peroxidation and antioxidant status: the effect of dietary luteolin on 1,2-dimethylhydrazine challenge. Cellular and Molec. Biology Letters, 10, 535, 2005.
9. Karpischenko A.I.: Medicynskie laboratornye tehnologii. Spravochnik. Sankt-Petersburg 2002.
10. Skrzydlewska E. et al.: Lipid peroxidation and ontioxidant status in colorectal cancer. World J. Gastroen., 11, 403, 2005.

SUMMARY

The levels of protein carbonyl groups, thiobarbituric acid reactive substances (TBARS), reduced glutathione, and glutathione-dependent enzymes, and c-kit expression in intestinal mucosa of rats after treatment with maleimide derivative 1-(4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1H-pyrrol-2.5-dione (MI-1) in doses 0.027 and 2.7 mg/kg have been studied. Colon cancer was induced by 1,2-dimethylhydrazine (DMH). The increase of the level of TBARS and protein carbonyl groups and glutathione antioxidant system in DMH-treated rats was shown. MI-1 restores changes of the studied parameters and blocks c-kit expression in intestinal mucosa of DMH-treated rats.

Keywords: maleimide derivative, 1,2-dimethylhydrasine, colon cancer, oxidative stress

STRESZCZENIE

Określono poziomy grup karbonylowych białek, produktów peroksydacji reagujących z kwasem tiobarbiturowym (TBARS), glutationu zredukowanego, enzymów zależnych od glutationu, i ekspresję c-kit w śluzówce jelit szczurów po podaniu pochodnej maleimidu 1-(4-Cl-benzyl)-3-Cl-4-(CF3-phenylamino)-1H-pyrrol-2.5-dionu (MI-1) w dawkach 0,027 and 2,7 mg/kg m.c. Raka okrężnicy indukowano przy użyciu 1,2-dimetylohydrazyny (DMH). Wykazano wzrost poziomów TBARS i grup karbonylowych białek oraz glutationowego systemu antyoksydacyjnego u szczurów, którym podawano DMH. Zastosowanie MI-1 powodowało cofnięcie się opisanych zmian w oznaczanych parametrach i blokowało ekspresję c-kit w śluzówce jelit szczurów.

Słowa kluczowe: pochodna maleimidu, 1,2-dimetylohydrazyna, rak okrężnicy, stres oksydacyjny