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LYLO V. V.[1], KARPOVA I. S.[1], KOTSARENKO K. V.[1], MACEWICZ L. L.[1], RUBAN T. A.[1], DZIUBA G. V.[2], LUKASH L. L.[1]

[1] Institute of Molecular Biology and Genetics of Natl. Acad. Sci. of Ukraine, Ukraine, 03680, Kyiv, Akad. Zabolotnogo str., 150, e-mail: lukash@imbg.org.ua

[2] Taras Shevchenko National University of Kyiv, Ukraine, 01601, Kyiv, Volodymyrska str., 64, e-mail: avdziuba@gmail.com

ISOLECTINS FROM *SAMBUCUS NIGRA* FLOWERS AND THEIR EFFECT ON *MGMT* AND P53 PROTEINS AMOUNT IN HUMAN CELLS *IN VITRO*

Lectins are carbohydrate binding proteins with wide spectrum of functions. Among the most significant known functions of lectins there are those involved in cell signaling, intercellular interactions, induction of cell cycle arrest and apoptosis, etc., and especially their participation in different defence mechanisms. Nowadays, lectins are in the focus of attention as natural compounds modulating drug sensitivity of tumor cells [1].

The main role of O6-methylguanine-DNA methyltransferase (*MGMT*) in the restore of primary DNA damages caused by alkylating compounds is well established [2]. Another key player in the system of cell defence from xenobiotics is an oncosupressor p53 [3]. There are some papers demonstrating the interrelation between these two proteins [4]. For example, it was shown [5] that MGMT induction by ionizing radiation did not occur in p53-deficient mice; the *MGMT* level was also shown to be lower in ovarian tumors with a wild type p53 than in tumors with a mutant one. Authors supposed that p53 downregulated the basal *MGMT* expression. On the contrary, in [6] the stable transfection of a *MGMT*-negative human cell line having a wild type *TP53* with a vector encoding *MGMT* was shown to increase p53 expression.

In our previous works [7, 8] the influence of *S. nigra* flower lectins on mutagenesis in mammalian cells *in vitro* was demonstrated. And in our other works [9, 10] the *S.nigra* bark lectin was shown to modulate *MGMT* gene expression in concentration dependent manner in human cells *in vitro*. However influence of lectins on DNA repair system is still one of the poorest studied phenomenon.

The aim of this work was to characterise *S. nigra* flower isolectins in the aspect of their effect on *MGMT* and p53 protein amount in a cell culture in order to reveal putative protective and anticancer potentials of these biologically active substances.

Materials and methods

In the experiments the standard line of larynx carcinoma cells Hep-2 was used. Total cell amount was estimated by direct counting in a hemocytometer. Different preparations of *S. nigra* lectins were used: a commercial bark lectin SNA-I (Lectinotest, Lviv, Ukraine) and two isolectins (named P1 and P2) obtained from water extracts of dried flowers by a modified method of isoelectric focusing [11] with resulting precipitation with 50% ethanol. Hemagglutinating activity (HAA) was estimated by adding 2% suspension of native human erythrocytes to serial two fold dilutions of the lectins in microtiter plates [12]. The lowest concentration of the lectin where the HAA was still observed was estimated. The carbohydrate specificity of these isolectins was tested with 7 sugars by inhibition of HAA. The Hep-2 cell culture was treated with lectins in a concentration 20 μg/ml during 8h with 16h postincubation. Conditions of cell treatment and protein extract obtaining were described previously [13]. SDS electrophoresis of the proteins was performed in a 12% polyacrylamide gel by the Laemmli method [14]. The total protein concentration was determined by the Bradford method [15]. Changes of MGMT and p53 amounts in cells were detected by Western blot analysis. Monoclonal antibodies against human *MGMT* (clone 23.2, isotype IgG2b, Novus Biologicals, USA) and p53 (clone BP53-12, Sigma, USA) were used in Western blot analysis with β-actin and stained membrane dencitometry as loading controls [16]. In our previous works [13, 17] it was revealed that used monoclonal anti-MGMT antibodies detected not only a *MGMT* protein at ≈24 kDa but also a protein at ≈48 kDa which was named MARP (anti-Methyltransferase Antibody Recognizable Protein). Statistical analysis was performed using Origin 8.1.

Table

Carbohydrate specificity of *S.nigra* flower isolectins P1, P2 and P3

Sugar	Minimal HAA concentration, mM		
	P1	P2	P3
Ramnose	25.000	—	100
Galactose	<0.006	0.781	0.7812
Galactosamin	<0.006	0.781	0.7812
Lactose	<0.006	0.781	1.5625

Results and discussion

Fractions obtained by isoelectrofocusing of *S. nigra* flower extracts were divided into three groups according to their pI: cathodic (pH 1-2), intermedial (pH≈5) and anodic (pH>9). All these groups showed hemagglutinating activity that is the evidence of lectin presence. The major lectin component P1 was concentrated in a zone of pH about 5, two minor components P2 and P3 migrated to a cathode and an anode respectively. The preparations were ranged according to the minimal HAA concentration: P2 (6.5 μg/ml) >P1 (31.2 μg/ml) >P3 (125 μg/ml). Some differences in carbohydrate specificity were observed as well (see tab.).

P1 demonstrated very high specificity to Gal, GalNac and Lac with inhibition concentration under 6μM while such specificity of P2 and P3 was about 100 times lower (781 μM).

We studied proliferation of mammalian Hep-2 cell after the treatment with *S. nigra* lectin preparations (fig. 1). P3 was not included into further experiments by technical reasons.

The cell proliferation activity was not significantly changed after pretreatment with these lectins (20 μg/ml), but the opposite tendency was observed in the case of different *S. nigra* flower isolectins. Lectin concentration 20 μg/ml was chosen in accordance with our previous studies [9] where the enhancing effect of 20 μg/ml of *S. nigra* lectin SNA-I on *MGMT* gene expression was shown.

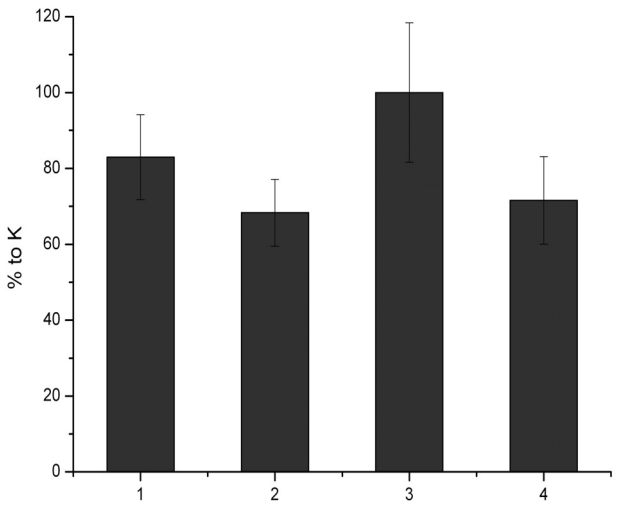

Fig. 1. Total cell number after pretreatment with *S.nigra* lectin preparations: 1 — intact control; 2 — P1; 3 — P2; 4 — SNA-I

Isolectins from *S. nigra* flowers demonstrated the same tendency of their influence on both the MGMT and MARP amount, but the severity of this effect depended on preparation used (fig. 2). At the same time, the p53 level was reduced under rhe P2 and SNA-I treatments.

Under the major isolectin P1 treatment the MGMT amount increased considerably exceeding the control level about 1/3 that is comparable with the effect of the commercial preparation SNA-I. It is interesting that the preparation P2 was even more effective in stimulation of MGMT expression (a half over the control level). In the case of MARP the similar tendency was shown but with much lover severity.

In the work [18] MGMT was shown to perform not only repair but also regulatory functions. As to MARP we assumed earlier [19] this protein to participate in the repair of cellular DNA damages caused by the action of complex alkyl groups. Different responce of MGMT and MARP on lectin effect may be the result of their involvement in different cellular signalling pathways.

At the same time *S.nigra* lectins demonstrated the opposite to MGMT and MARP tendency of their influence on the p53 level: isolectin P2 and SNA-I a little bit reduced the p53 amount. It is known that p53 and *MGMT* participate in cell defence particularly against O6-methylating drugs and radiation which are used in tumor therapy [20]. Lectins are known as regulators of many fundamental functions, but the mechanisms of their action on repair processes are still poorly understood. Obtained results give us the reason to suppose that exogenic *S.nigra* flower isolectins (which are highly specific to galactose) may use the own lectin-dependent regulatory pathways of a host organism, especially those which galectins

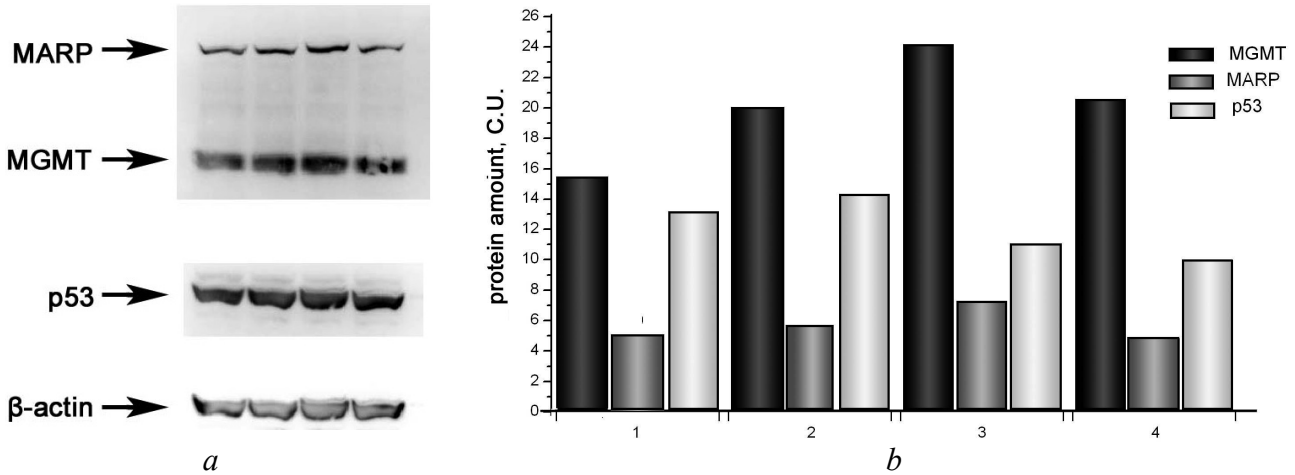

Fig. 2. Effect of *S. nigra* flower isolectins and bark lectin on the amount of MGMT, MARP and p53 protein in human cells *in vitro* (*a* – results of Western blot-analysis; *b* — results of densitometry of signal, corrected with the loading control): 1 — intact control; 2 — P1; 3 — P2; 4 — SNA-I

use [21]. Another suggestion based on data obtained is that *MGMT* was a primary lectin target, and p53 level changes were the consequence of the MGMT modulation.

It is assumed that the various lectins isolated from the same source may be elements of a same regulatory system [22] which may be sinergic, autonomic or antagonistic. That's why the studying of *S.nigra* flower isolectins having various physicochemical properties and carbohydrate specificity was the issue of our special interest. In fact, isolectins P1 and P2 with different pI and affinity to galactose demonstrated different effect on levels of all the proteins studied.

Conclusions

Variety of molecular forms of *S.nigra* flower lectins may explain the differences of their effects we observed. All lectins studied enhanced the amount of *MGMT* and, in a less extend, MARP proteins in human cell culture, but the minor P2 preparation being more efficient. Effect of lectins on p53 amount was opposite, and was observed only after cell treatment with P2 and SNA-I preparations.

It is supposed that *S. nigra* flower isolectins may use the own lectin-dependent regulatory pathways of mammalian cells. *MGMT* is likely to be the primary target of lectins, and the p53 level may be affected as a result of *MGMT* modulation.

LITERATURE

1. Ferriz-Martinez R.A., Torres-Artega I.C., Blanco-Labra A., Garcia-Gasca T. The role of plant lectins in cancer treatment / New Approaches in the Treatment of Cancer (ed. by C. Vazquez et al.).— NY.: Nova Sci, 2010.— P. 71–90.
2. Kaina B., Fritz G., Coquerelle T. Contribution of O6-alkylguanine and N-alkylpurines to the formation of sister chromatid exchanges, chromosomal aberrations, and gene mutations: new insights gained from studies of genetically engineered mammalian cell lines // Environ. Mol. Mutagen.— 1993.— 22.— P. 283–292.
3. Ozaki T., Nakagawara A. Role of p53 in Cell Death and Human Cancers // Cancers (Basel).— 2011.— 3, N 1.— P. 994–1013.
4. Grombacher T., Eichhorn U., Kaina B. p53 is involved in regulation of the DNA repair gene O^6-methylguanine-DNA methyltransferase (MGMT) by DNA damaging agents // Oncogene.— 1998.— 17, N 7.— P. 845–851.
5. Hengstler J.G., Tanner B., Möller L., Meinert R., Kaina B. Activity of O6-methylguanine-DNA methyltransferase in relation to p53 status and therapeutic response in ovarian cancer // Int. J. Cancer.— 1999.— 84.— P. 388–395.
6. Patyka M., Abdulkarim B., Xu Y., Sabri S. Cross-talk between the O (6) -Methylguanine-DNA methyltransferase (MGMT) and p53 in glioblastoma multiforme. // Molecular Cancer Therapeutics.— 2013.— 12, Suppl. 11.— C2–C2.
7. Lukash L.L., Karpova I.S., Miroshnichenko O.S., Tikhonova T.N., Lylo V.V., Man'ko V.G., Sukhorada E.M., Golynskaia E.L. The effect of the lectin from Sambucus nigra inflorescences on spontaneous and alkylating agent-induced mutagenesis in mammalian somatic cells // Cytol. Genet.— 1997.— 31, N 5.— P. 52–60.
8. Karpova I.S., Lylo V.V., Macewicz L.L., Kotsarenko K.V., Palchykovska L.G., Ruban T.O., Lukash L.L. Lectins of Sambucus Nigra as Biologically Active and DNA-protective Substances // Acta Hort. (ISHS).— 2015.— N 1061.— P. 93–102.

9. Macewicz L. L., Lylo V. V., Karpova I. S., Kotsarenko K. V., Ruban T. A., Lukash L. L. Plant and animal lectines as modulators of MGMT and MARP gene expression in vitro // Factors in experimental evolution of organisms.— 2014.— 15.— P. 260–264.
10. Lylo V. V., Karpova I. S., Kotsarenko K. V., Macewicz L. L., Ruban T. O. Lukash L. L. Lectins of *Sambucus nigra* in Regulation of Cellular DNA-protective Mechanisms. // Acta Hort. (ISHS).— 2015.— N 1061.— P. 103–108.
11. Sova O. Autofocusing — a method for isoelectric focusing without carrier ampholytes // J. Chromatography.— 1985.— 320.— P. 15–22.
12. Lutsyk M. D., Panasyuk E. N., Antonyuk V. A. Methods of lectine carbohydrate specificity investigation (the manual).— Lvov, 1983.— 22 p.
13. Lylo V. V. Matsevich L. L. Kotsarenko E. V. Babenko L. A., Kornelyuk A. I., Sukhorada E. M., Lukash L. L. Activation of gene expression of the O 6-methylguanine-DNA-transferase repair enzyme upon the influence of EMAP II cytokine in human cells *in vitro* // Cytol. Genet.— 2011.— 45, N 6.— P. 373–378.
14. Laemmli U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4 // Nature.— 1970.— N 227.— P. 680–685.
15. Bradford M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. // Anal. Biochem.— 1976.— 72.— P. 248–254.
16. Aldridge G. M., Podrebarac D. M., Greenough W. T., Weiler I. J. The use of total protein stains as loading controls: an alternative to high-abundance single protein controls in semi-quantitative immunoblotting // Journal of Neuroscience Methods.— 2008.— 172, N 2.— P. 250–254.
17. Kotsarenko K. V., Lylo V. V., Macewicz L. L., Babenko L. A., Kornelyuk A. I., Ruban T. A., Lukash L. L. Change in the MGMT Gene Expression under the Influence of Exogenous Cytokines in Human Cells *in vitro* // Cytol. Genet.— 2013.— 47, N 4.— P. 202–209.
18. Chahal M., Xu Y., Lesniak D., Graham K., Famulski K., Christensen J. G., Aghi M., Jacques A., Murray D., Sabri S., Abdulkarim B. MGMT modulates glioblastoma angiogenesis and response to the tyrosine kinase inhibitor sunitinib // Neuro Oncol.— 2010.— 12, N 8.— P. 822–833.
19. Kotsarenko K. V., Lylo V. V., Ruban T. P., Macewicz L. L., Kornelyuk A. I., Chernykh S. I., Lukash L. L. Influence of EMAP II, IFN-α2b and its medicinal preparations on the MGMT protein amount in human cells *in vitro* // Biopolym Cell.— 2014–30, N 6.— P. 448–453.
20. Bocangel D., Sengupta S., Mitra S., Bhakat K. K. p53-Mediated down-regulation of the human DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT) via interaction with Sp1 transcription factor // Anticancer Res.— 2009.— 29, N 10.— P. 3741–3750.
21. Rapoport E. M., Kurmyshkina O. V., Bovin N. V. Mammalian galectines: structure, carbohydrate specifity and functions // Biokhimija.— 2008.— 73 (4).— P. 483–497.
22. Lakhtin V. M., Afanasiev S. S., Aleshkin V. A., Nesvizhskij U. V., Lakhtin M. V., Shubin V. V., Cherepanova Yu. V., Pospelova V. V. Classification of the lectins as universal regulatory molecules in biological systems // Proc. Russ. Acad. Med. Sci.— 2009.— N 3.— P. 36–42.

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[1] Institute of Molecular Biology and Genetics NASU,
Ukraine, 03680, Kyiv, Zabolotnogo str., 150, e-mali: lukash@imbg.org.ua

[2] Taras Shevchenko National University of Kyiv,
Ukraine, 01601, Kyiv, Volodymyrska str., 64, e-mail: avdziuba@gmail.com

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Aim. To characterise *S.nigra* flower isolectins in the aspect of their effect on MGMT and p53 proteins amount. ***Methods.*** Lectin preparations P1, P2 and P3 with defferent pI were obtained by isoelectric focusing. The Hep-2 cells were treated with *S. nigra* lectins: P1, P2, and a commercial SNA-I. Changes of protein amounts were detected by Western blot analysis. ***Results.*** Isolectins were characterized accoding to their carbohydrate specificity. They increased MGMT amount with different severity. p53 level under the P2 and SNA-I treatments reduced. These lectins were shown to effect the amount of MARP protein as well. ***Conclusions.*** All lectins studied enhanced the amount of MGMT and MARP proteins. Effect of lectins on p53 amount was the opposite. MGMT is supposed to be the primary target of lectin action which uses own lectin-dependent regulatory pathways of mammalian cells, and p53 level may be affected as a result of MGMT modulation.

Keywords: *Sambucus nigra* lectins, protein level, MGMT, MARP, p53, human cell culture.