CELL BIOLOGY

The influence of lectins of different origin on mutagenic process of mammalian somatic cells in vitro

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Lectins of different origin can influence proliferation of human and Chinese humster cells in vitro, this effect may be compared with the action of cytostatic agent mitomicyn C. We have demonstrated that Sambucus nigra bark lectin controls genetic stability of mammalian somatic cells (hprt locus), in dose dependent manner reaching the maximum at the concentration of 20-200мg/ml. Significant increase in gene mutation frequency in the hprt locus, inhibition of cell proliferation and cytotoxic action were observed in the range of protein concentration from 2 to 2000 мg/ml. Probably, the influence of plant lectins (which belong to the RIP-protein family) on cell proliferation is not linked to their carbohydrate specificity but depends on the specific interactions of the lectin chain A with ribosomal proteins of the recipient cell which provide or hinder lectin associated N-glycosidase activity. Also, we have found that Perca fluviatilis roe lectin affects cell proliferation in the same way as plant RIP lectins.

Key words: lectins, RIP proteins, genetic stability, inhibition of prolifer ation, cytotoxic action, mammalian cells in vitro.

Introduction. Lectins are common carbohydrate-binding proteins of non-immunoglobulin nature. Biological functions of these proteins are various and depend on the structural and functional features of bio-systems, though it is considered that these functions are determined mainly by their ability to interact with carbohydrates, i.e. by their substrate specificity.

Protein-carbohydrate interactions take place in various biological processes. For example, these interactions can mediate the transmission of biological information [1, 2]. Also, exogenous lectins can influence such processes as adhesion, migration, apoptosis, and proliferation through the interaction with glycolysed surface of cell membrane, which contains numerous receptors. In addition, lectins are shown to affect spontaneous and induced mutagenesis in pro- and eukaryotic systems [4, 7].

Thus, the lectins, due to their natural occurrence and ability to influence basic matrix processes, are of great interest for the research aimed at their further usage as the modulators of spontaneous mutagenesis [8].

The objective of our work was to investigate the influence of lectins, different in their origin and carbohydrate specificity, on proliferation and genetic stability of mammalian cells *in vitro*.

Materials and Methods. In our experiments we have used the plant (*Sambucus nigra* bark, *Lens culinaris* seeds) and animal (*Persa fluviatilis* roe) lectins, received from

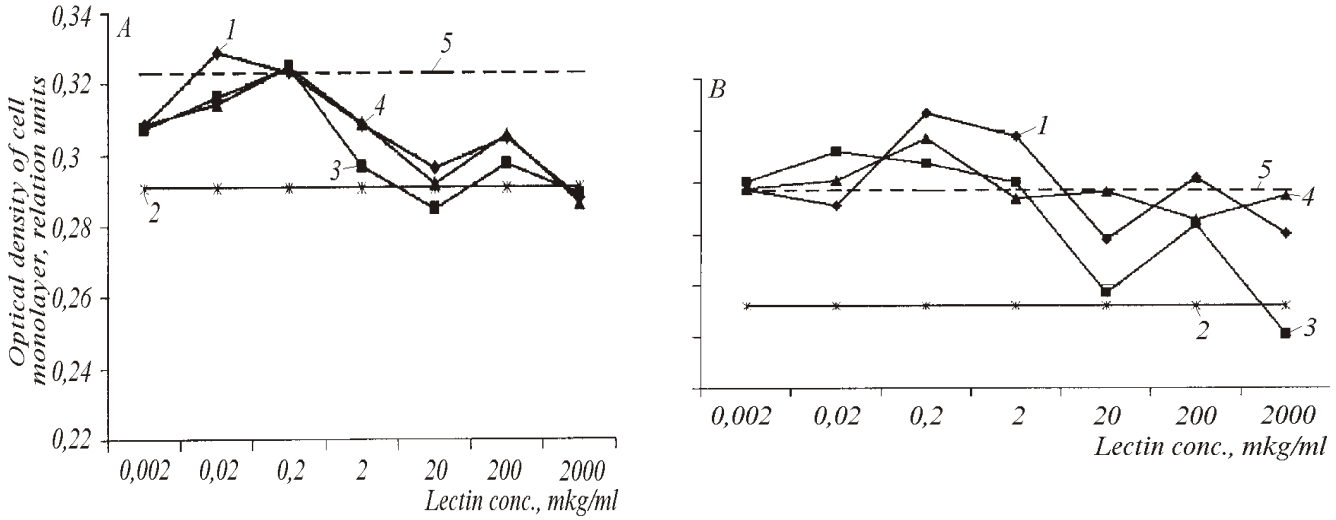

Fig.1 – The influence of lectins on proliferation of Chinese hamster cells (average results of 3 experiments); the influence of lectins on proliferation of human cell A-102 (average results of 3 experiments).

NVK Lectinotest, Lviv. These lectins differ not only in origin but also in molecular masses, which are 140, 49 and 100kDa respectively, and carbohydrate specificity. Thus, the *Sambucus nigra* lectin is specific to D-galactose, M-acetyl-D-galactosamine and sialic acid residues; the *Lens culinaris* lectin – to Ь-D-glucose and Ь -D-mannose. According to the modern classification of lectins from higher plants, both proteins belong to legumes superfamily [9] and to so called ribosome inactivating proteins (RIP-proteins) [10].Also the *Persa fluviatilis* lectin (NVK Lectinotest in-house project), is known to be specific to Ь -fucose, but currently no data are available regarding its classification and possible effect on mammalian cells.

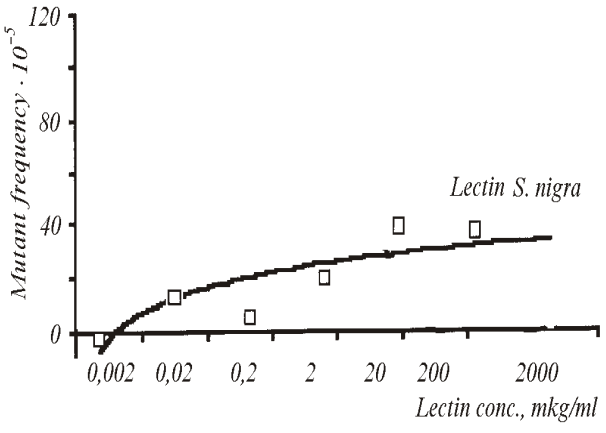

Fig.2 – The dependence of induction of resistant to 6MP clones on the *S.nigra* bark lectin concentration.

All experiments were performed *in vitro* using Chinese humster cells, line Blld-ii-FAF28C1237, and human cells, line A-102 (kindly provided by Dr. McCormick). The cells of both lines were used to determine the influence of the investigated proteins on the proliferation rate by a micro-culture method. The line Blld-ii-FAF28C1237, which is sensitive to the analogues of purine basis, was used to determine the influence of the proteins on the spontaneous mutagenic process. Due to such sensitivity, this test-system can be an invaluable tool for the induction of gene mutations in the *hprt* locus. The selection of mutant clones was performed for 12-14 days in Eagle's medium adding 6-mercaptopurine (6MP), at concentration of 60мg/ml. The experimental procedure was described in details earlier [11].

The study on influence of all investigated lectins on cell proliferation was performed according to the following scheme: the cells were treated with lectins for 4hours, the protein concentration was of 0.002, 0.02, 0.2, 2, 20, 200 and 2000мg/ml in Eagle's medium without serum. Cells incubated in Eagle's medium without serum and any lectins were used as a negative control. The cell division was inhibited by known cytostatic mitomicine C in concentration of 10мg/ml, (positive control). Fixation, cell staining and analysis of the results were performed after 43-44 hours of incubation.

The lectin of *S.nigra* bark was used only in experiments, where the induction of gene mutations in the *hprt* locus was studied. The treatment with the lectin lasted for 3

The dependence of number of clones resistant to 6MP on frequency of the S. nigra lectin concentration (μg\ml)

Variants	Concentration	Selection N, thousand	Cloning effectiveness	Resistant clone		Frequency, deducting control 10^{-5}	ρ
				Quantity	Frequency, 10-5		
1. Control	-	300	0,456	26	19,00	-	-
Lectin	0,002	250	0,814	33	16,21	-2,79	<0,05
	0,02	250	1,0	79	31,50	12,50	<0,01
	0,2	250	0,993	59	23,77	4,77	<0,05
	2	200	0,244	19	38,93	19,93	<0,001
	20	200	0,247	43	58,03	39,03	<0,001
	200	300	0,178	30	56,18	37,18	<0,001
2. Control	-	200	0,916	3	1,67	-	<0,01
Lectin	0,002	200	0,628	23	18,31	16,64	<0,01
	0,2	200	0,453	7	7,73	6,06	<0,01
	20	200	0,820	56	34,15	32,48	<0,01
	2000	200	0,772	42	27,20	25,53	<0,01
MNNG	0,5	200	0,399	43	53,88	52,21	<0,01

Comments:
1. Cell treatment duration in experiment 1 - 3 hours;
2. Cell treatment duration in experiment 2 - 4 hours;
3. Mutation expression in experiment 1-3 days;
4. Mutation expression in experiment 2-4 days;

and 4 hours in different experiments. As a positive control in these experiments the cells were treated with N-methyl-N-nitro-N-nitrosoguanidine (MNNG), in concentration of 0.5мg/ml for 1 hour. The mutagenic effect of this factor has been studied earlier [12].

Results and Discussions. The influence of lectins on proliferation. Our results have revealed that the *S.nigra*, *L.culinaris* and *P.fluviatilis* lectins inhibit proliferation of Chinese humster cells in the concentration of 2-2000мg/ml similar to mitomicine C (Fig. 1). The decrease in the cells number revealed by microtest may be caused not only by inhibiting proliferation, but also by cytotoxic effect of lectins on the cells. The results of these three experiments are shown in Fig.1. We have found that the inhibition of cell division and cytotoxic action of all lectins tend to weaken (decrease) with the decreasing of their concentration (up to 0.2мg/ml). But at lectins concentration of 0.002мg/ml the inhibition of proliferation and/or cytotoxic effect are increased again. More detailed study of the proteins action at low concentrations may confirm the existence of less expressed peak of the inhibition activity.

Thus, the Chinese humster cells test has revealed significant similarity among different types of lectins in their biological effect.

Similar correlation between the inhibiting and cytotoxic effects of lectins from *L.culinaris* seeds, *P.fluviatilis* roe and concentration of these proteins was observed in the system of human cells A-102 (Fig.2). The strongest inhibiting effect of the *L.culinaris* lectin in this system appeared at the concentrations of 20 and 200мg/ml.

Thus, the *P.fluviatilis* roe lectin retained a relatively high mitogenic effect at the concentrations of 0.2 and 2мg/ml. All lectins did not reveal any biological effect at the concentrations of 0.002мg/ml. The Human cells responded to the *S.nigra* bark lectin only at concentration of 0.2 and 200мg/ml by increasing cell proliferation (Fig.2).

The Cytotoxicity of lectins is the result of typical enzymatic activities, well known today, but for some RIP proteins at high concentrations non-canonical enzymatic characteristics (DNAse and RNAse) are described. It is known that cytotoxicity of the *L.culinaris* and *S.nigra* lectins is the consequence of the ribosome deadenylation

(basic function of RIP proteins). The fact that the proteins with different carbohydrate specificity affected equally the proliferation of Chinese humster cells indicates that the effect depends most likely on the level of N-glycosidase activity of the lectin A-chain intracellular processes. It does not contradict with the concept that carbohydrate specificity of lectins is important only on the first stages of protein-cell interaction, namely recognition and binding while the RIP toxicity depends on the specific interactions of lectin A-chain with ribosomal proteins of the recipient cell [10, 13]. For example, it is known that ricin highly active towards ribosomes of yeast and mammals and not plants and bacteria ribosomes. The majority of RIP proteins of the first type have wider interaction spectrum comparing to RIP proteins of the second type, which manifest RNA N-glycosidase activity to the animal ribosomes preferably. This difference depends on ribosomal proteins, which hinder or stimulate binding the lectin A-chain to the sarcine/ricin loop of rRNA, the sequence of which is conservative. For example, proteins L9 and L10 from rat liver are specific to the ricin A-chain [13].

This difference exactly seems to explain the absence of toxic effect of the *S.nigra* lectin on human cells contrary to its action on Chinese humster cells.

The influence of lectins on mutagenesis. It is of great interest to compare inhibiting and/or cytotoxic effect of lectin with its possible influence on genetic stability in mammalian cells. This study was performed using the induction of recessive mutations as a model system. The *S.nigra* bark lectin was chosen as a mutagenic agent since it is well studied [4, 7]. It strongly inhibited the proliferation of Chinese humster cells in the concentration of 2-2000мg/ml.

To induce gene mutations in the *hprt* locus the *S.nigra* lectin varied in the wide range of concentrations (Table 1, Fig.3). All protein concentrations (except 0.002мg/ml in experiment 1) caused the increase in number of clones resistant to 6MP. The lectin induced the clone resistance with the frequency much higher than in control experiment, this difference being statistically significant. Maximum mutagenic effect was observed at the protein concentration of 20мg/ml, the further concentration increase does not change the frequency of mutagenesis, which reaches the plateau (Fig.3). Similar data on the lectin from *S.nigra* raceme have been published earlier[7]. Probably, the mutagenic effect of the *S.nigra* lectins does not depend on their carbohydrate specificity.

The comparison of induced mutations frequencies while investigating biological and chemical factors in experiment 2 revealed that the effect of the lectin at the concentration of 200мg/ml did not exceed the frequency of the mutagenesis induced by supermutagenic factor MNNG at the concentration of 0.5мg/ml.

It is likely, that the biological action of lectins (mutagenic effect, inhibition of proliferation and cytotoxic effect) is not connected with their carbohydrate specificity but depends on the level of N-glycosidase activity of the lectin A-chain. As to the RIP proteins, that investigated plant lectins belong to, the difference in biological action in two different model systems may be explained different specificity of ribosomal proteins of the recipient cell which determines binding of the lectin A-chain with sarcine/ricin loop of rRNA. However, further investigations are necessary to prove this assumption.

Conclusion. The lectins of different origin and substrate specificity can influence proliferation of somatic mammalian cells *in vitro*, the effect is comparable with the action of chemical mutagens. The inhibition of proliferation and/or cytotoxic effect of the lectins are shown at the concentrations of 2-2000мg/ml. The *P.fluviatilis* roe lectin was revealed to affect the proliferation of mammalian cells *in vitro* similar to plant RIP proteins.

We demonstrated the direct concentration dependence of the *S.nigra* lectin influence on genome stability, namely statistically significant increase in gene mutation frequency in the *hprt* locus. At the protein concentration over 20мg/ml the mutagenic effect reaches plateau.

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Влияние лектинов разного происхождения на мутационный процесс в популяциях соматических клеток млекопитающих in vitro

Резюме

Исследованные лектины разного происхождения способны влиять на пролиферацию соматических клеток человека и китайского хомячка. Их эффекты сопоставимы с действием цитостатика митомицина С. Обнаружена прямая зависимость влияния лектина коры бузины черной Sambucus nigra на генетическую стабильность соматических клеток млекопитающих от концентрации белка. Статистически достоверное повышение частоты генных мутаций по локусу hprt, а также ингибирование и/или цитотоксическое действие наблюдались в диапазоне концентраций белков 2-2000 мкг/мл. При концентрациях белка 20-200 мкг/мл проявлялся эффект насыщения. Вероятно, влияние растительных лектинов, относящихся к RIP белкам, на пролиферацию культур клеток не связано с их углеводной специфичностью, а зависит от структурно-функциональных особенностей системы лектин-клетка. Получены новые данные о способности лектина икры окуня Persa fluviatilis изменять пролиферацию клеток млекопитающих в сравнении с действием растительных RIP белков.

Ключевые слова: лектины, RIP белок, генетическая стабильность, ингибирование пролиферации, цитотоксическое действие, клетки млекопитающих в культуре.

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