CHAPTER 10

BIOTECHNOLOGY AND BIOENGINEERING: BACTERIAL LECTINS AS COMPONENTS OF ORIGINAL MEDICINAL PRODUCTS OF BIOLOGICAL/BIOTECHNOLOGICAL ORIGIN

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INTRODUCTION

Carbon-binding proteins (lectins) have attracted the interest of researchers over the past decades due to their functional role and diverse applications. Lectins are biologically active substances present in every biological system and every living organism. They participate in carbohydrate-protein recognition processes and the identification of biological objects. Lectins have found application in medicine. Their unique properties allow them to be used to study the structure and properties of carbon-containing biopolymers, influence metabolic processes, and act as analytical reagents. Lectins are expensive reagents because they are often sourced from exotic, hard-to-find raw materials. Finding unconventional lectin sources and isolating lectins with unique carbohydrate specificity remain challenging tasks. Technologies have been developed for producing plant lectins and their conjugates, as well as lectins of animal origin. Lectins have been found in all living organisms – from viruses and bacteria to mammals. Microbial lectins offer advantages over lectins of other origins, as they can be enhanced using genetic engineering, have a wide range of origins – from bacteria to fungi, possess high activity, and exhibit a wide variety of specificities[1, 2].

Bacterial lectins are the least studied. Lectins are formed during the life cycle of microorganisms. The functions of microorganisms can

[1] Kovalenko E.A. Lectin biosynthesis by bacteria of genus Bacillus depending on their physiological functions. 11-th Int. Lectin Conf. Tartu Univer. Est. Acad. Sci: Abstr. Tartu. 1989. P. 40.

[2] Krivan H.C. Bacterial adhesions: identification of glycolipid receptors for many pulmonary pathogens. Glycoconjugate J. 1988. 5(3). P. 351.

be explained by the specific interaction of lectins with carbohydrate-containing polymers[3, 4].

Most lectins are located intracellularly, while some are found on the cell surface or in the extracellular growth medium. Based on their location, lectins are divided into two main groups: bound or associated lectins (membrane-bound, cytoplasmic, intracellular) and unbound or extracellular lectins (soluble, extracellular). Bound-form lectins are involved in adhesion processes and have been found in pathogenic, opportunistic and saprophytic microorganisms. The group of extracellular lectins includes bacterial toxins (botulism, dysentery, diphtheria, and others). These are toxic proteins that have carbon-binding properties, lack agglutinating activity, and act as pathogenicity factors. The biological reactions that lectins participate in occur due to the presence of specific recognition sites in their molecules and can precipitate polysaccharides, glycoproteins, and glycolipids. The following biological reactions of lectins have been studied more thoroughly: enzymatic activity and toxicity to eukaryotic cells, phagocytic activity, inhibition of cell growth, agglutination of erythrocytes and mitogenic stimulation of lymphocytes. Among lectins, a group of bifunctional lectins is distinguished, which are represented by enzyme and toxin lectins. An example of an enzymatic bacterial lectin is the extracellular lectin of Vibrio cholerae with proteolytic activity. This group includes bifunctional lectins, in which the substrates for the lectin and enzyme are various carbohydrates. Toxic lectins include extracellular lectins: enterotoxins, tetanus and botulinum toxin, as well as Shigella toxins. Unlike true lectins, they do not have agglutinating activity and are monovalent with one active binding site.

These reactions are divided into two types: primary (the interaction of lectins with carbohydrate receptors on the cell surface) and secondary (metabolic transformations within the cell and its response to the lectin). Lectins are proteins or glycoproteins that selectively bind to carbohydrates. In medical practice, lectins are used mainly in diagnostics and as objects of promising research. The direct use of lectins as drugs is limited due to their high toxicity. Lectins have been used to create drugs used in anticancer therapy – toxic lectins (from mistletoe, ricin), which are capable of selectively blocking protein synthesis in tumor cells, as immunomodulators and immunosuppressants, as antiviral agents, for targeted drug delivery. Experiments are being conducted with lectins from B. subtilis bacteria to increase the effectiveness of chemotherapy. Some lectins (concanavalin A) have immunosuppressive effects and are used to minimize rejection during organ transplantation. Plant lectins (snowdrop lectins) show

[3] Kundu M., Basy Y. Chakrabarti P. Purification and characterization of an extracellular lectin from Mycobacterium smegmatis. FEBS Lett. 1989. 256. N 1-2. P. 207–210.

[4] Mandal C. Sialic acid binding lectins. Experientia. 1990. 46. P. 433–441.

activity against herpes viruses and coronaviruses. They block their reproduction at early stages. Lectins are considered as vectors in nanocomposites for precise drug delivery to affected tissues[5].

1. Biological properties of lectins

Primary lectin reactions include cell agglutination and adhesion. Agglutination results from the cell's surface charge in the presence of acidic carbohydrates, such as sialic acids. Agglutination reactions are based on the selective binding of a lectin to a carbohydrate receptor on the cell surface.

The lectin acts as a ligand in this reaction. This requires the formation of a bond between the active lectin receptor and the corresponding receptor on the sensitive cell. Inhibition of agglutination by the carbohydrate confirms the presence of the carbohydrate structure on the cell surface, to which the lectin is specific. A lectin's agglutinating ability is possible due to the presence of two active binding sites in its molecule. Monovalent lectins more often block receptors and do not cause cell agglutination. For many bacterial lectins, optimal agglutination values are within the neutral and acidic pH range. For most bacterial lectins, temperature has no effect on the reaction. Hemagglutination occurred at 10, 30, and 42°C. In the presence of chelating agents, lectins undergo conformational changes in the molecule and are unable to cause cell agglutination. Modifications that alter the valence or size of the lectin have been described. For example, treatment of bean agglutinin with glutaraldehyde increased the lectin's hemagglutinating activity against human erythrocytes by 100–200 times. The nature of hemagglutination is influenced by the surface properties of agglutinating cells, receptor sensitivity, membrane fluidity, and other factors. Treating agglutination-sensitive cells with enzymes – glucosides or carbohydrate-modifying agents – learns information about the nature and properties of lectin receptors. Cells that are not agglutinated by low concentrations of lectins become agglutinable. A study of the hemagglutinating activity of the B subunit of E. coli enterotoxin revealed that, in the absence of treatment of human erythrocytes with agents, only group B erythrocytes were agglutinated by lectin. Glycosidases act on agglutination. The degree of agglutination of eukaryotic cells by Shiga toxin has been described when treated with enzymes. This lectin toxin is specific to oligosaccharides. Treatment of erythrocytes with neuraminidase resulted in the absence of the hemagglutination reaction, which is caused by lectins, such as sialo-specific lectins[6].

[5] Miller R.L. Properties of a sialic acid-specific lectin from the slug Limax flavus. Meth. Enzym. 1987. 138. P. 527–536.

[6] Muller H.E. Neuraminidases of bacteria and protozoa and their pathogenetic role. Behr. Inst. Mitteilungen. 1974. 55. P. 34–56.

In some experiments, neuraminidase increased the interaction between red blood cells and lectins. Moreover, the high density of sialic acids on the cell surface imparts a negative charge and can prevent agglutination. Terminal sialic acids on intact red blood cells in humans and animals often prevent toxin binding. Red blood cells are treated with neuraminidase. This promotes the cleavage of some terminal sialic acids and the release of galactose residues, which leads to enhanced hemagglutination.

Agglutination of animal red blood cells is a differential indicator used to identify individual lectins in microorganisms with lectin systems.

Bacterial surface lectins bind to various types of phagocytic cells (human neutrophils, mouse peritoneal macrophages). The lectinophagocytosis model is based on lectin receptor reactions.

The result of such interactions is phagocytosis of bacteria. Three types of interactions have been described: a membrane-integrated macrophage lectin receptor on the bacterial surface, a soluble lectin receptor on the bacterial cell surface, and a soluble bacterial lectin receptor on the macrophage (Figures 1, 2)[7, 8, 9].

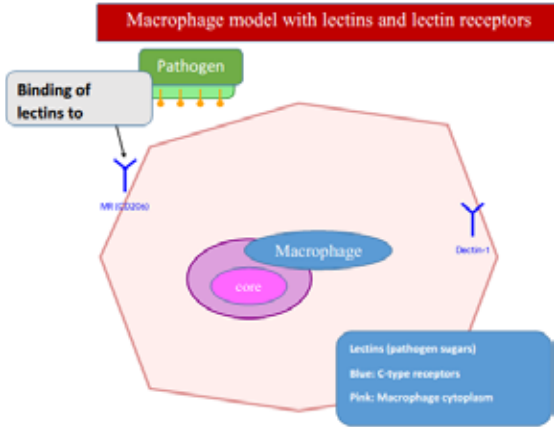

Fig. 1. Model of macrophage with bacterial lectins and lectin receptors

Source: Author.

[7] Wang Q., Zhang L., Hu W., Hu Z.H., Bei Y.Y., Xu J.Y. et al. Norcantharidin-associated galactosylated chitosan nanoparticles for hepatocyte-targeted delivery. Nanomed. 2010. 6. 371e81.

[8] Jain N.K., Jain S.K. Development and in vitro characterization of galactosylated low molecular weight chitosan nanoparticles bearing doxorubicin. AAPS Pharm. Sci. Tech. 2010.11. 686e97.

[9] Suriano F., Pratt R., Tan J.P., Wiradharma N., Nelson A., Yang Y.Y. et al. Synthesis of a family of amphiphilic glycopolymers via controlled ring-opening polymerization of functionalized cyclic carbonates and their application in drug delivery. Biomaterials. 2010. 31. 2637e45.

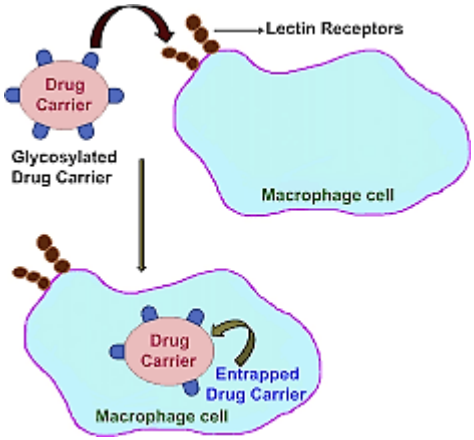

Fig. 2. Lectin receptor-mediated targeting to macrophages

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Sepsis is a condition caused by various organisms such as bacteria, fungi, and viruses, which can lead to inadequate blood flow, organ failure, and death due to the body's immune response. Diagnosing sepsis is challenging because its symptoms are often associated with other pathologies. Bloodstream infections are linked to high morbidity and mortality, and the economic cost of complications associated with sepsis is also quite high**.** The gold standard method for pathogen detection is classical culture method. This method has several disadvantages: the long duration it takes, the high volume of blood required (20–30 mL for each repeat, 3–4 repeats), the potential for false-negative results due to the need to start treatment immediately. Disadvantages of classical culture methods, research into new detection methods is ongoing. Various techniques have been developed: nucleic acid-based sensors, polymerase chain reaction (PCR) and its derivatives, and mass spectrometry sensors. The design of biosensor systems has made it possible to detect various nucleic acids. Immobilizing different DNA-modifying enzymes on a substrate surface has enabled the detection of pathogenic bacteria and viruses. [10] PCR has revolutionized biological research. The development of qPCR after the invention of conventional PCR (qPCR) has expanded the potential of molecular methods. Molecular methods are accurate, rapid, versatile and capable of detecting microorganisms in a variety of samples

[10] Esra Ekiz, Emine Kubra Tayyarcan, Eylul Evran, Kubra Guven, Esra Acar Soykut, Ismail Hakki Boyaci. Investigation of the effect of bacteriophage cocktail on microbial quality in the case of cold chain breakage: a case study on Escherichia coli contamination in milk. Food and Humanity. 2023. 1. P. 1073–1081. https://doi.org/10.1016/j.foohum.2023.09.004

(environment, products, clinical samples). A significant disadvantage of PCR is its inability to differentiate between living and dead cells. After cell death DNA remains intact for several days. Due to the inability to distinguish between living and dead cells, DNA-based diagnostics can lead to an overestimation of the number of living cells[11].

One approach is the use of reverse transcriptase PCR (RT-PCR) for mRNA detection. This can lead to false-positive results due to the high concentration of dead bacteria and the inability to remove mRNA during sample processing. mRNA tends to better indicate the presence of live cells compared to DNA detection. Another technique involves measuring the RNA/DNA molar ratio. It lacks sufficient sensitivity for detecting live bacteria in environments with high levels of dead bacteria. Cross-linking agents can reduce the PCR amplification signal of dead bacteria. Factors such as dye selection, dye concentration, incubation conditions, and the presence of many dead cells can also affect the results. Bacteriophages are viruses that disrupt the metabolism of bacteria in order to replicate. In the case of lytic phages, they cause the death of the host cell. Phages require bacterial host cells for replication and spread. This makes them obligate intracellular parasites. The first step in phage infection is the attachment of the phage to a bacterial cell, followed by the injection of the viral genome into the host cell[12, 13, 14].

The phage uses the metabolism of the host cell to replicate its genome and produce virions. The virions cause lysis of the bacterial host cell and spread into the environment, completing the lytic cycle. A method for the detection of E. coli, a model bacterium, using bacteriophage-based lysis without a DNA extraction step, combined with direct qPCR, is proposed. The mechanism by which bacteriophages selectively bind to their host cells, attach to living bacteria, lyse the host, and replicate is exploited. The objective is to quantify live bacteria present in a plasma matrix using bacteriophages. This method aims to provide a rapid (3 h), accurate and reproducible method for the detection of live pathogenic cells in a plasma matrix with minimal

[11] Mayra C. García-Anaya, David R. Sepulveda, Alma I. Sáenz-Mendoza, Claudio Rios-Velasco, Paul B. Zamudio-Flores, Carlos H. Acosta-Muñiz. Phages as biocontrol agents in dairy products. Trends in Food Science & Technology. 2020. 95. P. 10–20.

[12] Na Li, Xiaoming Yuan, Chun Li, Nuo Chen, Juan Wang, Bo Chen, Shubo Yu et al. A novel Bacillus cereus bacteriophage DLn1 and its endolysin as biocontrol agents against *Bacillus cereus* in milk. International Journal of Food Microbiology. 2022. 369. 109615. https://doi.org/10.1016/j.ijfoodmicro.2022.109615.

[13] Yen-Ling Fang, Chih-Hung Wang, Yi-Sin Chen, Chun-Chih Chien, Feng-Chih Kuo, Huey-Ling You, Mel S. Lee and Gwo-Bin Lee. An integrated microfluidic system for early detection of sepsis-inducing bacteria. Lab. on a Chip. 2021. 1.

[14] Merve Calimci, Tugba Tezcan, Emine Kubra Tayyarcan, Kubra Guven, Ismail Hakki Boyaci, Ugur Tamer. Bacteriophage-based live bacteria detection for rapid infection diagnosis. Talanta. 286. 127569.

sample volume. This eliminates the need for complex pre-treatment and DNA extraction steps[15, 16, 17, 18].

Mitogenic stimulation of lymphocytes is one of the properties of lectins. Lymphocytes are the only cells in living organisms that can recognize antigens and initiate immunological reactions. Lymphocyte activation is a model for studying the body's response to specific antigens. For a mitogenic response to occur, lectin binding to carbohydrate sites on the lymphocyte cell surface is essential. In response to lectin, lymphocytes produce and secrete protein factors that exhibit mitogenic activity. After treatment with lectin-mitogens, lymphocytes undergo blast transformation, increase in size, develop cytoplasmic vacuoles, and stimulate metabolic processes – the synthesis of DNA, proteins, RNA, and lipids. The transport of potassium, calcium, and other ions increases. Cell division occurs. This process continues in the presence of lectin and then becomes independent of it. The mitogenic activity of lectins is demonstrated in relation to native lymphocytes. Other lectins can induce lymphocyte mitosis after treatment of the cell surface with neuraminidase. Some lectins induce the synthesis and release of lymphokines, which are polyfunctional substances, by lymphocytes. Lymphokines influence cell growth, regulate immunological reactions, and have a cytotoxic effect.

A number of lectins have been described as having the ability to induce the production of interferon by lymphocytes. Interferons are unique substances, low-toxic natural bioregulators and chemotherapeutic agents. Two types of interferon have been described: type 1 includes leukocyte (α) and fibroblast (β) interferons (induced by viruses and their nucleic acids); type 2 includes γ-interferon (induced by mitogens, lectins, and antigens).

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[15] Коваленко Е.О. Позаклітинні лектини бактерій роду Bacillus: автореф. дис. на здобуття наук. ступеня док. біол. наук: спец. 03.00.07 «Мікробіологія» / Е.О. Коваленко. – Київ, 1999. – 36 с.

[16] Пат. 68373 Україна, МПК7 C12P19/04 (C12P19/04, C12 R1:125). Застосування сіалоспецифічного лектину, виділеного із штаму Bacillus subtilis 668 IMB для інгібіції репродукції вірусу імунодефіциту людини / Підгорський В.С., Рибалко С.Л., Коваленко Е.О., Шарикіна Н.І., Гетьман К.І., Максименок О.В., Іванська Н.В.; заявник та власник патенту Інститут мікробіології і вірусології ім. Д.К. Заболотного НАНУ. – № 2000095282; заявл. 14.09.00; опубл. 16.08.04, Бюл. № 8.

[17] Пат. 83305 Україна, МПК C12P 19/04 (A61K 39/245, A61P 1/16). Лікарський засіб, який містить бактеріальний лектин для лікування хворих, уражених гепатитом С / Підгорський В.С., Коваленко Е.О., Рибалко С.Л., Шарикіна Н.І., Гетьман К.І.; заявник та власник патенту Інститут мікробіології і вірусології ім. Д.К. Заболотного НАНУ. – № а 2006 13193; заявл. 13.12.06; опубл. 26.06.08, Бюл. № 12.

[18] Пат. 83578 Україна, МПК A61K 38/16 (A61P 31/16, C12P 19/04). Лікарський засіб, який містить бактеріальний лектин для лікування хворих, уражених грипом / Підгорський В.С., Коваленко Е.О., Рибалко С.Л., Шарикіна Н.І., Гетьман К.І.; заявник та власник патенту Інститут мікробіології і вірусології ім. Д.К. Заболотного НАНУ. – № а 2006 13194; заявл. 13.12.06; опубл. 25.07.08, Бюл. № 14.

A separate group of lectins are bifunctional lectins: enzyme lectins and toxin lectins. A classic example of a bacterial lectin with enzymatic properties is the extracellular lectin of Vibrio cholerae, which has proteolytic activity. This group includes bifunctional lectins, in which the substrates for the lectin and enzyme are different carbohydrates. Lectin-enzymes do not chemically modify carbohydrates. Examples of bifunctional lectins include glucose- and mannose-specific lectins with α-galactosidase activity from Vicia faba and Lens culinaris.

A property of bacterial lectins is their toxicity to eukaryotic cells. Toxic lectins include extracellular lectins (bacterial exotoxins): enterotoxins, tetanus and botulinum toxins, and Shigella toxins. These are toxic proteins with carbohydrate-binding properties. Unlike true lectins, they do not possess agglutinating activity and are usually monovalent lectins with a single active binding site (Figures 3, 4).

Toxins exhibit image and tissue specificity when interacting with a susceptible host. This specificity is based on the presence of complementary receptors on the surface of the host cell recognized by the toxin. This recognition is stereospecific. It represents the interaction of a soluble toxic ligand and an insoluble cell surface receptor.

In the universal model of the structure of lectin-like toxins, the toxic complex consists of three domains: the A-domain is enzymatic and is responsible for the activity of the toxin, the B-domain is binding and determines the specific binding of the toxic complex to the host cell, and the E-domain is an independent component and is responsible for the penetration of the toxin

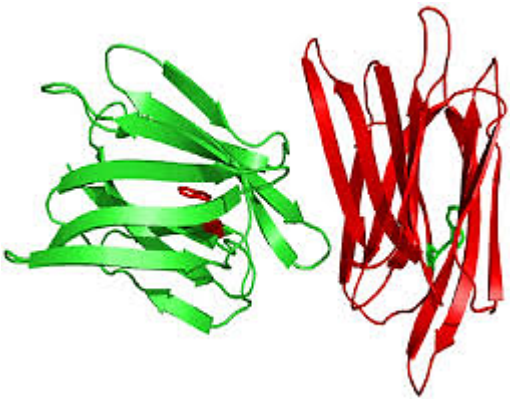

Fig. 3. Ribbon structure of a banana lectin (2BMY) generated from PyMol. Trp residues are shown in both subunits

Source: DOI: 10.1371/journal.pone.0062428.g001.

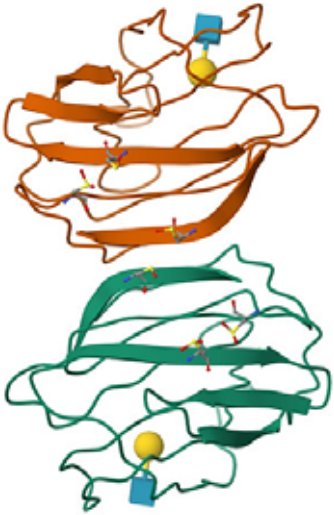

Fig. 4. Structure of S-Lectin

Source: DOI: https://www.rcsb.org/structure/1SLT.

into the cell. Conventionally, bacterial toxins are divided into groups: protein synthesis inhibitors; toxins that activate nucleotide cyclase; toxins with an unknown mechanism of action.

Diphtheria toxin from Corynebacterium dyphtheriae is an example of a toxin that inhibits protein synthesis. The toxin affects cells in three stages: the enterotoxin binds to the surface of the eukaryotic cell (the B domain is responsible for the specific binding of diphtheria toxin to the cell surface); fragments of the toxin penetrate the cell cytoplasm (where protein synthesis is inhibited); and polypeptide elongation factor 2 is inactivated. Lectins specific for mannosyl- and N-acetyl-glucosaminyl oligosaccharides were found to antagonize this toxin's common glycoprotein target. In the presence of Con A and WGA, cells were protected from the toxin's action and actively synthesized the protein. Lectins that bind galactose and N-acetylgalactosamine were not antagonists of the toxin.

Shigella toxin, which is secreted into the environment by Shigella dysenteriae, is a protein synthesis inhibitor. The toxin contains two components: the A chain (molecular weight 32,225 D) and the B chain (molecular weight 7,691 D). Shigella toxin exhibits biological properties, including enterotoxicity, neurotoxicity, and cytotoxicity. It differs from diphtheria toxin in that it does not

affect soluble cytosolic factors in protein synthesis processes. The toxin's action leads to irreversible inactivation of ribosomes.

The toxin's activity on tissue culture cells can be inhibited by treating the cells with protease, isozyme, or by adding specific carbohydrate inhibitors – chitotriose and chitotetrose. Shigella toxin binds actively to the glycolipid globotriosylceramide.

2. Bacterial lectins as components of original pharmaceutical compositions

The properties of extracellular lectins of the Bacillus family, namely, sialospecific lectin isolated from the Bacillus subtilis 668 IMB strain for inhibiting the reproduction of the human immunodeficiency virus, were studied. A drug containing bacterial lectin has been developed for the treatment of hepatitis C, influenza, and herpes types 1 and 2. To create the original pharmaceutical composition, previously synthesized heterocyclic compounds with a bis-structure were used, which have proven themselves in studies as antitumor agents. To create molecular mixtures of bis-uracil derivatives with an original pharmacophoric group and lectins, the most active producers of extracellular lectins from the Ukrainian Collection of Microorganisms of the IMV NASU were selected, namely, bacterial lectins of saprophytic strains Bacillus subtilis 668 IMV and Bacillus polymyxa 102 KSU[19, 20].

Determination of acute toxicity in two animal species (different routes of administration) showed that the bacilli lectins are moderately and slightly toxic substances (Table 1). The experimental animals were white non-linear male mice (body weight 17.0±2.0 g and 22.0±2.0 g) and male rats (body weight 160.0+20.0 g).

The bis-derivative 5-FU (I) was used as a chemical component to create a new pharmaceutical composition[21, 22] (Figure 5).

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[19] Пат. 84928 Україна, МПК A61K 38/16 (C12P 19/04, A61P 31/22). Лікарський засіб, який містить бактеріальний лектин для лікування хворих, уражених герпесом 1 і 2 типів / Підгорський В.С., Коваленко Е.О., Рибалко С.Л., Шарикіна Н.І., Гетьман К.І.; заявник та власник патенту Інститут мікробіології і вірусології ім. Д.К. Заболотного НАНУ. – № a 2006 13192; заявл. 13.12.06; опубл. 10.12.08, Бюл. № 23.

[20] Пат. 34108A Україна, МПК 6 C07D239/00, C07C21/00. Спосіб отримання 1,1'-(2"-бром-2"-хлоретенил)-біс-(5-фторурацил)-у, який має протипухлинну активність / Вельчинська О.В.; заявник та власник патенту Інститут фармакології та токсикології АМН України. – № 99063049; заявл. 02.06.1999; опубл. 15.02.01, Бюл. № 1.

[21] Welchinska, E. & Vilchynska, V. (2016). New compound N1,N1′-(2"-bromo-2"-chloroethenyl)-bis-(5-fluorouracil) as the active antitumor agent for sarcoma 180. CBU International conference proceedings 2016: Innovations in Science and Education, 4, 740–743. DOI 10.12955/cbup.v4.842.

[22] Welchinskaya, H.V., Piecuszak, B., Kovalenko, E.A., Sharykina, N.I., Getman, K.I. & Podgorsky, V.S. (2003). Biological activity of bacterial lectins and their molecular complexes with heterocyclic bis-adducts. Mikrobiolohichnyy zhurnal – Journal of Microbiology (Kiev, Ukraine: 1993). 65(5), 20–25.

Table 1

Acute toxicity parameters of Bacillus lectins by different routes of administration

Routes of administration	Lectin 668, LD50, mg/kg	Lectin 102, LD50, mg/kg
Mice		
Intramuscular	68 (46-101)	294 (210-318)
Subcutaneous	71 (59-84)	248 (195-301)
Intraperitoneal	89 (75-106)	200 (154-246)
Intravenous	37 (26-54)	–
Rats		
Intraperitoneal	71 (62-80)	60 (52-68)
Intravenous	52 (45-59)	–

Note: Here and further, total preparations of lectins 668 and 102 were investigated.

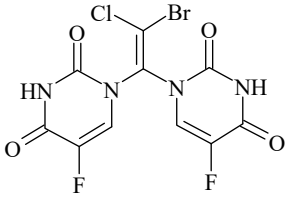


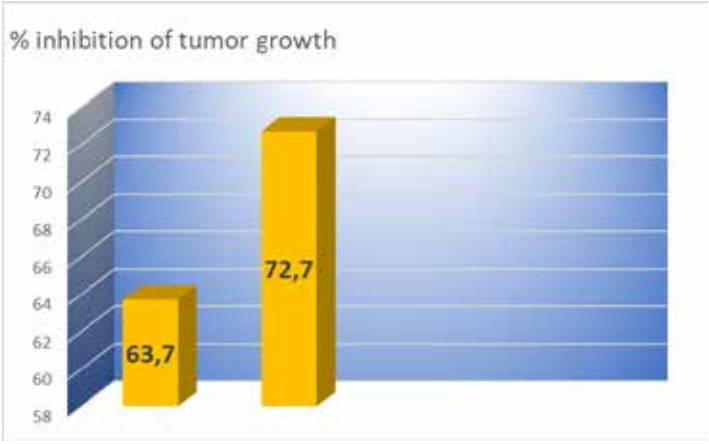
Fig. 5. Structure of compound I

LD50, mg/kg of compound I – 125 mg/kg. The bis-derivative of 5-FU I and its molecular mixtures are less toxic than lectin 668, but more toxic than lectin 102. When more toxic substances (bis-derivative I and lectin 668) were combined, the overall toxicity of the molecular mixture decreased by 1.06 times. The antitumor activity of the obtained molecular mixture of compound I with bacterial lectins was assessed by the following indicators: % inhibition of tumor growth; % death of experimental animals.

Studies on the specific antitumor activity of the molecular mixture Compound I-Lectin were conducted on models of experimental tumor growth Plis lymphosarcoma at doses of 24.0 mg/kg, 31 mg/kg, 35 mg/kg (comparative drug 5-FU). A pronounced antitumor effect with a high % inhibition of tumor growth was registered on Plis lymphosarcoma in mixtures: lectin 102 – bis-derivative of 5-fluorouracil (I) (% inhibition of tumor growth – 63.7%). It was found that when replacing the lectin component – lectin 102 in the molecular mixture with lectin 668 in Plis lymphosarcoma, a higher % inhibition of tumor growth was recorded.

For the mixture: lectin 668 – bis-derivative 5-FU (I) this value was 72.7% – 1.14 times higher % inhibition of Plis lymphosarcoma growth than in the mixture: lectin 102 – bis-derivative 5-FU (I).

Combining more active drugs in Plis lymphosarcoma: lectin 102 (% inhibition of tumor growth – 50.0%) and bis-derivative 5-FU (I) (% inhibition of tumor growth – 75.3–91.07%) in the molecular mixture did not lead to an increase in % inhibition of tumor growth (63.7%) (Figure 6).



Lectin 102-Compound I Lectin 668-Compound I

Fig. 6. Study of the antitumor activity of molecular mixtures of 5–FU and Lectins 102, 668on lymphosarcoma Plis

To confirm the composition and structure of the synthesized compound (I) and its molecular mixtures with bacterial lectins, elemental analysis, PMR spectroscopy, chromatographic and spectral methods were used. The high-performance liquid chromatography (HPLC) method, which was previously successfully implemented in the analysis of biologically active substances, was actively introduced into the analysis of the obtained substances[23, 24, 25].

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CONCLUSIONS

Bacterial lectins have specific pharmacological properties and are an exclusive object for the creation of new medicines of biological and biotechnological origin using their participation as a biological component. The parameters of acute toxicity and antitumor activity of a separate chemical component – bis-derivative 5-FU (I) and bacterial lectins 102 and 668, as well as their molecular mixtures, were investigated. When comparing the acute toxicity parameters of molecular mixtures of bis-derivative 5-fluorouracil with lectin 102 or lectin 668, it was found that the molecular mixture of bis-derivative 5-FU with lectin 102 is 1.01 times more toxic than the second (LD50 135 mg/kg and 137 mg/kg, respectively). A pronounced antitumor effect with a high % tumor growth inhibition was registered on Plis lymphosarcoma in the mixture of lectin 102 – bis-derivative of 5-fluorouracil (I) (% tumor growth inhibition – 63.7%). When replacing the lectin component – lectin 102 in the molecular mixture with lectin 668 in Plis lymphosarcoma, a higher % tumor growth inhibition was recorded – 72.7% – 1.14 times higher % growth inhibition of Plis lymphosarcoma than in the mixture: lectin 102 – bis-derivative of 5-FU (I). The obtained molecular mixtures of Lectin-Compound I are highly effective in the treatment of tumors and are of interest in the creation of promising drugs.

SUMMARY

Based on the conducted research and the obtained results, a conclusion can be drawn regarding the effectiveness of using bacterial lectins as a biological component for the creation of medicines of biological/ biotechnological origin. Bacterial lectins are an exceptional object for the creation of new drugs of biological and biotechnological origin. Molecular mixtures based on the chemical component – bis-derivative 5-FU (I) and bacterial lectins 102 and 668 were created. The parameters of acute toxicity of molecular mixtures were studied. Their significant antitumor activity on the tumor strain Lymphosarcoma Plis was revealed. An increase in antitumor activity was found when replacing lectin 102 (% tumor growth inhibition – 63.7%) with lectin 668 (% tumor growth inhibition – 72.7%) in the molecular mixture. Bacterial lectins are unique objects of research in the creation of new molecular mixtures with specific pharmacological activity. Bacterial lectins are a group of chemicals that are not fully studied to date and require more attention with the prospect of creating original pharmaceutical compositions – drugs of the future.

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