БИОЛОГИЧЕСКИЕ НАУКИ

BIOPREPARATION FOR *IN SITU* ANTITUMOR VACCINATION

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Abstract. The present study is focused on the first attempt to use an enzymatically produced biological preparation of cyclic diguanosine monophosphate (cyclic di-GMP) for the therapy of animal cancer. Feline breast carcinoma was chosen as the test model. The preparation was administered intratumorally to induce the immunogenic death of a part of the cancer cells and thus carry out the so-called *in situ* antitumor vaccination. Preliminary results indicate good therapeutic prospects of studied biopreparation for animal cancer treatment.

In conclusion, the expedience of further trials of cyclic di-GMP preparation for *in situ* antitumor vaccination was stated. The need to supplement this mono-preparation with another immunostimulating adjuvant characterized by a mechanism of action distinct from that exhibited by cyclic di-GMP was emphasized. DNA preparation comprising the so-called immunostimulating CpG motifs was provided as an example of such compound.

Аннотация. Настоящее исследование посвящено впервые предпринятой попытке использовать ферментативно полученный биопрепарат циклического дигуанозинмонофосфата (цикло-ди-ГМФ), для терапии рака животных. В качестве животной модели была выбрана карцинома молочной железы кошек. Препарат вводили внутриопухолево в расчете вызвать иммуногенную гибель части раковых клеток и таком образом, осуществить так называемую противоопухолевую *in situ*-вакцинацию. Предварительные результаты указывают на перспективность использования исследованного биопрепарата для терапии онкологических заболеваний животных.

В заключении была подчеркнута целесообразность дальнейшего изучения препарата цикло-ди-ГМФ для противоопухолевой *in situ*-вакцинации. При этом особо отмечена необходимость дополнить этот моно-препарат еще одним иммуностимулирующим соединением, характеризующимся механизмом действия, отличным от такового, проявляемого цикло-ди-ГМФ. В качестве примера такого соединения предложен препарат ДНК, содержащий так называемые иммуностимулирующие CpG-мотивы.

Keywords: Veterinary medicine, cats, immunotherapy, cyclic diguanosine monophosphate, therapeutic *in situ* antitumor vaccine.

Ключевые слова: Ветеринария, кошки, иммунотерапия, циклический дигуанозинмонофосфат, терапевтическая *in situ*-противоопухолевая вакцина.

Introduction. Recent years have seen the tremendous progress of immunotherapy as a novel highly promising trend in treatment of various diseases, including infectious, autoimmune and oncopathologies. This approach envisages mobilization of inherent protective shield of human organism – the immune system to counter hostile bioattack.

Adjuvants – the compounds stimulating immune response of the body act as potent therapeutic agents [1]. Lately numerous new types of adjuvants were identified and further used in experiments aimed at development of anticancer vaccines [2].

One of such immunity promoters is cyclic dimeric guanosine-5’-monophosphate (cyclic-di-GMP). This dinucleotide discovered in late 1980s finds ubiquitous distribution among bacterial species, in contrast to mammals. Bacterial cyclic di-GMP plays the role of multipurpose secondary messenger coordinating diverse aspects of microbial growth and behavior, like motility, formation of biofilms, cell division and serves

as one of the key factors responsible for expression of virulence in bacteria [3, 4].

It is generally recognized nowadays that apart from the vital molecular signal function, cyclic di-GMP may also be released by bacteria to interact with immune system of humans and animals and modulate host response [5, 6]. Cyclic di-GMP was shown to be able to activate innate potential of many immunocytes, including dendritic cells, macrophages and monocytes [7].

Cyclic di-GMP molecules secreted by bacteria or liberated in the course of cell lysis perform the part of PAMPs (pathogen-associated molecular patterns) and hence could be recognized by native immune system of humans and animals with the aid of receptor proteins, like most cited STING (stimulator of interferon genes) [8] and DDX41 [9]. Upon activation STING induces generation of interferons of the I type and antiinflammatory cytokines essential for development of congenital and adaptive immune response [10].

So far plentiful evidence has been stockpiled to support the assertion that STING protein plays a pivotal role in concentrating immune counter attack on tumor target [11–14]. This circumstance has focused considerable attention on elaboration of various chemically modified STING agonists (including cyclic di-GMP) for further use in cancer therapy.

The studies on spontaneous tumor regression [15, 16] and the inferences drawn from theoretical and practical heritage of Dr. W. Coley – the surgeon who practiced in New York over a hundred years ago and succeeded in full recovery of certain inoperable cancer cases [17], proved that curative effect was largely based on intratumor injection of immunostimulating drugs. According to modern concepts, direct delivery to neoplastic tissue of agents promoting immunity is capable to partially decompose tumor and turn it *in situ* into personalized vaccine [18–20].

Taking into account the above-mentioned aspects, we have attempted in the present investigation to use intratumor supply of cyclic di-GMP preparation for treatment of feline mammary gland carcinoma.

Experimental section. Research was conducted at facilities of Vitebsk municipal veterinary station and Vitebsk State Academy of Veterinary Medicine. Test specimen of cyclic di-GMP was produced by enzymatic procedure at laboratory of molecular biotechnology of Institute of Microbiology, NAS of Belarus using diguanylate cyclase enzyme isolated from recombinant bacterial strain [21].

The studies evaluating the impact of cyclic di-GMP on oncopathological process were carried out using mongrel cats with diagnosed mammary gland carcinoma (tumor size averaged 4 × 6 cm). 10 test animals with clinical manifestations of carcinoma (second-third stages of oncopathology) were engaged in the experiments. They were divided into 2 groups, each comprising 5 heads.

Sterile solution of cyclic di-GMP in concentration 5 mg/ml was injected into tumor site of sick animals during 4 treatments spaced by fortnight intervals at the dose 0.5 ml per head.

The animals were monitored throughout 2 months. Improved health status was recorded in the treated animals by the end of observation period. The cats demonstrated normal activity, their appetite was restored. By that moment tumor dimensions decreased by 20–25 %, falling down to 3.0–3.3 × 4.5–4.8 cm (Fig. 1). Noteworthy that following cyclic di-GMP treatment test animals showed indications for surgical intervention.

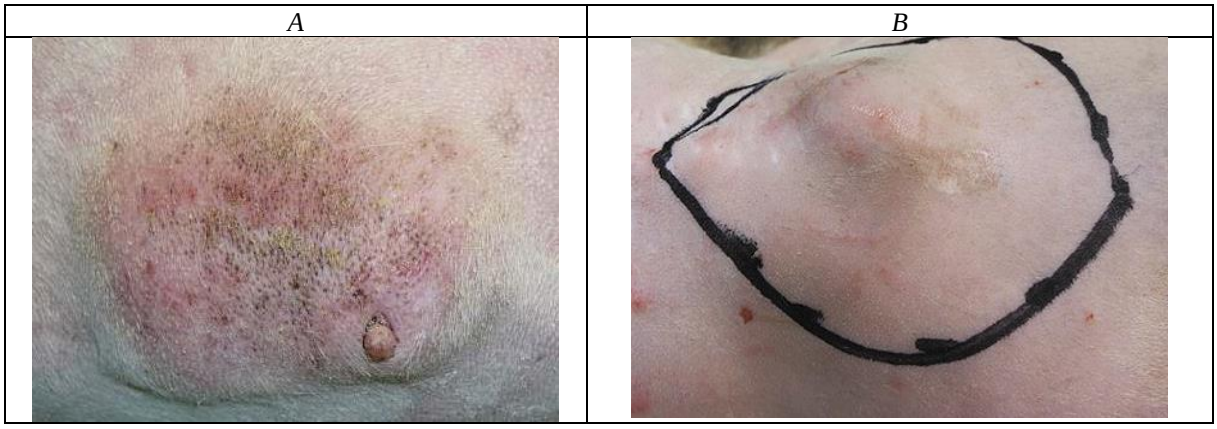

Fig.1. Condition of cat breast tumor prior to (A) and after (B) treatment

The control group (Fig. 2) not exposed to treatment was distinguished by general aggravation of symptoms and physical exhaustion while tumor size was not affected. In some animals tumor capsule was damaged, leading to leakage of internal contents. Overall outcome of pathological process was predicted as unfavorable.

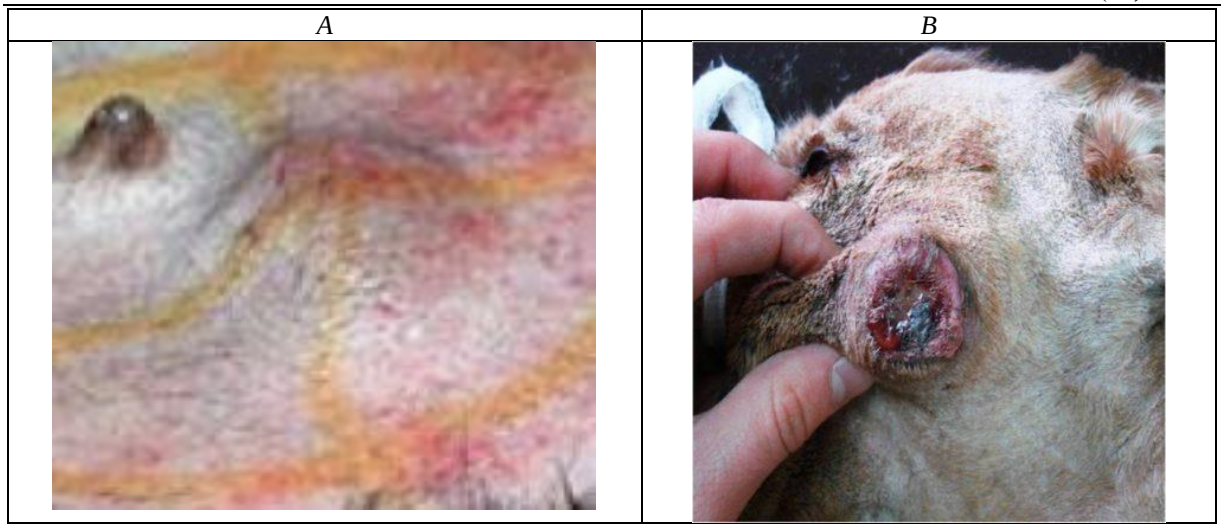

Fig. 2. Condition of the control feline breast tumor before (A) and after (B) the experiment

The obtained findings indicate encouraging prospects of cyclic di-GMP preparations for control of oncodiseases in animals. It appears expedient therefore to carry on trials of cyclic di-GMP preparations for *in situ* antitumor vaccinations both in the separate mode and in combination with other adjuvants characterized by different mechanism of action.

In this respect keen attention is aroused by DNA preparations containing the so-called immunostimulating CpG motifs [22–24].

CpG motifs are special 2'-oligodeoxynucleotide structures harboring in the central position of the molecule non-methylated CpG-dinucleotides.

Such molecular conformations are available in huge amounts in DNA of viruses, bacteria and other prokaryotes. It should be stressed that although eukaryotic DNA also incorporates certain levels of CpG-dinucleotides, it remains immunologically neutral because the majority of constituent CpG dinucleotides are methylated in C5-positios of cytosine residue.

Biological significance of CpG motifs is disclosed in the course of infections when these DNA components released from virions or bacterial cells provide an alarm signal to inborn human or animal immunity system and induce thereby swift defensive reaction.

Noteworthy that chemically synthesized 2'-oligodeoxynucleotides carrying non-methylated CpG-dinucleotides imitate bacterial DNA and display similar immunopromoting action.

Looking ahead, it may be stated that currently short synthetic single chain CpG-oligodeoxynucleotides (CpG-ODNs) are widely recognized as the golden standard of vaccine adjuvants capable to generate mighty antigen-specific antibody and Th1-celular immune responses in many vertebrate species, including humans.

In the body of human and animal hosts CpG-ODNs bind with peculiar cell receptor (TLR9) localized in dendritic cells, macrophages, natural killers and other antigen-presenting cells. Such binding initiates a cascade of molecular events triggering maturation, differentiation and proliferation of diverse immunocytes, e.g. B and T lymphocytes, NK cells and monocytes/macrophages. As a result cellular signal pathways are switched on leading to induced synthesis of numerous proinflammatory cytokines and chemokines and modulation of cellular inflammatory reaction. These properties enable CpG-ODNs to act as highly promising vaccine adjuvants and immunotherapeutic agents to combat infections, cancer and even allergies [25].

It should be noted that to date the most popular method of CpG-ODN production relies on multistage chemical synthesis and CpG motifs are applied mainly as synthetic ODNs comprising nuclease-resistant but toxic phosphorothioate internucleotide bonds.

In our opinion, more attractive strategy of producing preparations containing CpG-dinucleotides is the technique devised by the team of Canadian researchers implying engineering of recombinant bacterial strain capable to replicate and maintain plasmids enriched with CpG-motifs. Such biotechnological method of CpG-DNA synthesis possesses several advantages over chemical counterpart: the production process is more facile and will not require to resort to ecologically unfriendly solvents and reagents [26].

To promote this trend we engineered a recombinant Escherichia coli strain generating plasmid carrying the maximal number of CpG motifs among the described analogs (104 repeats of nucleotide sequence GTCGTT immunogenic for humans) [27].

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