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Review article

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THE USAGE OF TRANSGENIC ANIMALS IN THERAPEUTIC PROTEINS PRODUCTION

WYKORZYSTANIE ZWIERZĄT TRANSGENICZNYCH DO PRODUKCJI BIAŁEK TERAPEUTYCZNYCH

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Streszczenie. Możliwość tworzenia i wykorzystywania zwierząt transgenicznych może znacznie wpłynąć na wiele aspektów ludzkiego życia. Zwierzęta te mogą usprawnić medycynę i dać nowe możliwości przemysłowi farmaceutycznemu czy spożywczemu. Celem tej pracy jest przedstawienie dostępnych informacji o zwierzętach genetycznie zmodyfikowanych oraz możliwości ich wykorzystania w różnych dziedzinach życia i nauki. Przybliżono aspekty związane z wykorzystaniem zwierząt o zmodyfikowanym genomie jako bioreaktorów służących do produkcji białek stosowanych terapeutycznie. W pracy wyjaśniono pojęcie GMO, omówiono wykorzystanie zwierząt transgenicznych – korzyści oraz zagrożenia z tym związane. Skupiono się głównie na wykorzystaniu zwierząt o zmodyfikowanym genomie jako bioreaktorów do produkcji białek terapeutycznych, a także na rodzajach tych białek i źródłach ich pochodzenia.

Key words: therapeutic proteins, GMO, animals as bioreactors, transgenic animals.
Słowa kluczowe: białka terapeutyczne, GMO, zwierzęta jako bioreaktory, zwierzęta transgeniczne.

INTRODUCTION

The development of genetics and molecular biology has allowed researchers to not only familiarize themselves with the mechanisms governing evolution and inheritance, but also to look into the genetic code of all living organisms. Researches could also intervene in these mechanisms due to the increasingly advanced technologies. It was discovered that encoding of the corresponding proteins by organisms or preventing the formation of others may have major advantages, not only in terms of learning about their functions in the body.

The possibilities offered by the creation and use of transgenic animals are very broad and can significantly affect many aspects of human life. Biotechnology companies have no illusions that the use of such animals can improve medicine, or provide new opportunities for the pharmaceutical and the food industry.

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The aim of this paper is to provide knowledge about genetically modified animals and their use in various areas of life and science. The main objective of this paper is to present previous achievements of studies on transgenic organism in terms of the use of animals with modified genome as bioreactors for the production of therapeutic proteins.

TRANSGENIC ANIMALS

When attempting to define the term *transgenic animals*, one should first define the concept of a genetically modified organism (GMO). According to the Act of 22.06.2001 on microorganisms and genetically modified organisms, the term GMO is understood as an organism other than human, in which the genetic material was altered in a way that does not occur naturally by mating or natural recombination.

The most frequently applied modifying technique is a knock-out gene technique, which involves the extraction of a specific DNA sequence (Górska and Kowalski 1997). It results in the lack of synthesis of the protein encoded by the extracted gene, and thus a change in the functioning of the modified organism with respect to the unmodified organism. The *knock-out* technique makes is possible to insert new, exogenous genes in place of the extracted ones, using the phenomenon of homologous recombination.

Despite the existence of numerous techniques applied to modify animal genomes, it is not easy to modify them in a way that would enable the use of animals as bioreactors. It is impeded by, inter alia, low efficiency of integrating the transgene with the animal gene, as well as the fact that the integrated transgene is expressed only in 50% of cases. These problems result in a high price of transgenesis (Grzybowski 1998).

THE USE OF TRANSGENIC ANIMALS

The research on transgenic animals is carried out for scientific and practical purposes. The main objective of cognitive works is to gain knowledge about genetic mechanisms that control the physiological systems of humans and animals, and to create models of genetic diseases. Practical objectives include, inter alia, the improvement of quality when producing animals and the use of modified animals as bioreactors that produce substances designated for biomedical use (Linkiewicz and Sowa 2007).

The research on transgenic animals made it a lot easier for researches to become familiar with the processes occurring in humans. Modified animals are used as genetic models of human diseases, such as diabetes, dwarfism, cancer or cardiovascular diseases, and as objects of experimental tests aimed to create methods for treating these diseases. The research on transgenic mice provided a lot of information on the mechanisms of formation and development of tumors.

The diversity of available modifications of animal genomes made it possible for the transgenic animals to be used not only for cognitive purposes. Attention is also drawn to the possibility of using genetic modifications to obtain specific breeding characteristics in farm animals, such as improving the quality of meat and growth rate of carcass composition. It became possible to improve the processes and quality of dairy products by changing the protein composition of milk, which speeds up its production (Skrobiszewska 2011).

However, there are also modifications that aim at increasing the resistance of animals to parasites, such as cows resistant to prion diseases, including BSE (Linkiewicz and Sowa 2007). Consecutively, one could also mention improvements of the animal metabolism, better control over reproduction, and biomedical applications, at the head of which is the acquisition of therapeutic animal proteins and xenotransplantations of organs and the use of xenogeneic tissues and cells.

THE BENEFITS AND RISKS OF GENETIC MODIFICATIONS

When dealing with the production and breeding of transgenic animals one cannot ignore an important issue which involves its risks and benefits. Many advantages have already been mentioned earlier in this paper. The most important of these may include resistance to diseases, improvement of quality characteristics of food products, manufacture of therapeutic proteins, xenotransplantation of organs, or the development of model systems of human diseases (Stankiewicz 2007).

Although the above mentioned benefits of the production of transgenic animals may seem rational, this is only one side of the dispute. Issues related to GMO, and most of all transgenic animals, raise many ethical concerns. Their production also entails some risks that concern mainly environmental issues. One of the most important threats are still not entirely known side effects of the spread of modified animals in the natural environment, as it is virtually impossible to control this process (Stankiewicz 2007).

Moreover, one can also distinguish several equally important dangers that may be caused by increasing popularization of the use of genetically modified organisms (Żarski et al. 2009). Furthermore, one can notice that there is a potentially negative economic consequence of the distribution of animals with modified genome, because as in the case of transgenic plants, there is a risk of the monopoly of biotechnological corporations owning the rights to the specific modified strains. The most serious threat associated with the dissemination of transgenic organisms are the economic and social consequences, that can strike in the existing food producers and their dependence on companies owning proprietary organisms (Lisowska and Chorąży 2011). Additionally, researches emphasize the existence of risks arising from unknown long-term consequences of the consumption of food products derived from genetically modified animals and the possibility of allergic reactions (Żarski et al. 2009).

THE USE OF TRANSGENIC ANIMALS FOR THE PRODUCTION OF THERAPEUTIC PROTEINS

Drugs produced by biotechnological techniques, including therapeutic proteins, are increasingly used in pharmacology, since in many cases they show superior performance to the drugs produced by traditional chemical methods.

A branch of industry that uses transgenic animals as bioreactors for the production of therapeutic proteins has been developing more dynamically since 1987, when scientists succeeded in producing the first foreign protein in the mammary glands of modified animals. The main products of this industry are human proteins that are produced for medical needs.

The use of transgenic animals is easier than using the most common bacteria. It should be also mentioned that animal cells are able to produce proteins, whose production by microorganisms is not possible due to the lack of post-translational modifications of proteins (Akash et al. 2015).

SOURCES OF THERAPEUTIC PROTEINS

So far, scientists have managed to produce more than 900 different proteins in the organs of transgenic animals. Production occurs in the milk of cows, sheep and goats, blood, urine, semen and eggs. Forecasts predict that in future the main source of human therapeutic proteins will be mammary glands of modified animals (Linkiewicz and Sowa 2007).

Transgenic animals are used to produce large amounts of complex human proteins. Cows, sheep, goats, pigs and rabbits are used because of the high expression of transgenes, low maintenance costs and high reproduction rate (Nayak 2010). These animals are designed to direct the synthesis of proteins without adversely affecting the health of a particular animal.

Attention should be paid to many differences in animal production and microbiological human growth hormone (hGH). So far this hormone was produced only in complicated bacterial cultures, and the process itself was very slow. Meanwhile, in 2006, scientists managed to create a cow that produces human growth hormone in milk in the amount of 5 g/l (Tyczewska and Bąkowska-Żywicka 2008). It was calculated that 15 transgenic cows would be able to meet the entire global demand for the abovementioned hormone. A similar situation exists in the case of goats producing antithrombin, an anticoagulant given to people with impaired blood clotting. Only one transgenic goat can deliver antithrombin in amounts comparable to 90 000 human donors.

However, researches have noticed important deficiencies in the production of biopharmaceuticals using mammals. It was observed that transgenic mammals, in which the desired protein is produced by the mammary gland, often experience uncontrolled expression of foreign genes, which may result in serious malformations, as impaired fertility and gigantism (Bednarczyk et al. 2006). Furthermore, it was noticed that proteins encoded by transgenes produced by the mammary gland are not always subject to the correct post-translational modifications. It should also be noted that the acquisition of proteins from transgenic mammalian cells is conditioned by high production costs and the inability to produce more than a few kilograms per year (Houdebine 2009).

The solution to this problem might be the use of birds, especially White Leghorn hens (Bednarczyk et al. 2006). A valid argument for this theory is the production capacity of hens, which are able to make 330 eggs per year, where each contains up to 6.5 g of protein. An example can be the antibody of the IGg group, which can be obtained from hens in the amount comparable to 10 rabbits. In addition, the protein expression of hen eggs corresponds to only 7 genes. The disadvantage of the use of hens as bioreactors is hampered production, causes by a specific development cycle. The result is a complete uselessness of microinjection technique, which is the most common method of producing transgenic animals.

Another alternative to the use of mammary glands is the production of proteins in the semen of transgenic animals. The main advantage of using semen is its abundance and ease of acquisition, primarily in pigs. A single boar can give 200–300 ml of ejaculate, 2–3 times per week (Lipinski et al. 2006).

THE TYPES OF THERAPEUTIC PROTEINS OBTAINED FROM TRANSGENIC ANIMALS

The amount of biopharmaceuticals produced using genetically modified animals at the present time reaches almost a thousand, and it is predicted that in the future most of them will be produced in this way (Linkiewicz and Sowa 2007). Currently, therapeutic proteins play one of the most important roles in the production and development of pharmaceutical products (Akash et al. 2015).

The following section aims at defining some of the most important therapeutic proteins produced by transgenic animals.

1. Growth hormone (hGH) – the therapeutic that is used in the treatment of symptoms associated with its deficient. Growth hormone has been produced by a strain of *E. coli*, but scientists managed to create rabbits and cows, which may be a rich source of this protein.
2. Lactoferrin – the protein that participates in the fight against infections and inflammation of the eyes and lungs. It is also involved in immune responses. Its main functions are iron binding capacities and antibacterial properties. Currently, the main producers of lactoferrin are cows and goats.
3. Lysozyme – the protein with strong antibacterial properties, is an important factor preventing bacterial infections. It is used to limit the growth of intestinal bacteria. The main producers are transgenic goats and mice (Tyczewska and Bąkowska-Żywicka 2008).
4. Collagen – the protein that builds connective tissues, the main constituent of skin, tendons and bones. It is used to cover implants and produce artificial skin. It is also used as a filler in plastic surgery and a component of anti-wrinkle creams.
5. Erythropoietin – the hormone produced by liver and kidneys that stimulates erythropoiesis, that is red blood cell production. It is used in the treatment of anemia. Erythropoietin is obtained from a number of modified animals, including rabbits and pigs (Tyczewska and Bąkowska-Żywicka 2008).

SUMMARY

Production of therapeutic proteins and other organic substances applicable in the industry constitutes a very large part of the pharmaceutical market. The possibility of using transgenic animals has a significant impact on most of the production processes, which affect not only the speed and amount of the produced protein, but also its price.

Biotechnological processes allowing to obtain organisms with altered genes to obtain a specific and desired feature are constantly being improved. Such processes may lead to popularization and acceptance of the use of such organisms by a larger group of potential customers.

In spite of the occurring defects, stemming mainly from the inadequate knowledge of genetic mechanisms, the production of therapeutic proteins in the glands of transgenic animals is a very promising technique, demonstrating significant advantages over existing microbiological and chemical techniques. Taking into account the fact that it is still a young and continually improved field, it can be expected that the use of transgenic animals as bioreactors is the future of biopharmaceuticals production.

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Abstract. Ability to create and use of transgenic animals can have a significant impact on many aspects of human life. These animals can improve medicine and provide new opportunities for the pharmaceutical or the food industry. The aim of this work is to collect available information about genetically modified animals, and the possibilities of their use in various areas of life and science. Aspects which are related to the use of transgenic animals as bioreactors for the

production of proteins for therapeutic use will be discussed. The work will explain the concept of GMO, discuss the use of transgenic animals – what are benefits and risks that may be associated with it. The focus is primarily on the use of transgenic animals as bioreactors for the production of therapeutic proteins, the types of these proteins and their source of origin.