POSSIBILITIES AND RANGE OF GENE THERAPY

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There is a growing body of evidence that the observed characteristics in pharmacokinetics and pharmacodynamics of drugs are genetically determined and that they primarily include drug metabolism and transport processes. Current acknowledgements, based on collected evidence, clearly show that an early adjustment of therapy regime to genetic characteristics of patients may help to avoid side effects. Such an approach stands for an individualized, optimal therapeutic use of drugs, based on the dose regime adjusted to an individual genotype. Gene therapy is the intentional transfer of genetic material into human somatic cells in prophylactic, therapeutic or diagnostic purposes. While the gene transfer technology is very advanced, ethical principles related to this procedure are still the subject of discussion. The goal of gene therapy is to correct genetic defects and to establish a normal cell functioning. Most of the techniques of gene therapy should enable the replacement of defective genes by those that function normally. The ideal vector should influence specific target cells, without stimulating the inflammatory response in the host cell. In addition, it should facilitate the transfer of genes of different length (the length of the code gene therapeutic sequences vary considerably and there should be a possibility of inserting regulatory sequences in transduction and expression) and integrate into the host cell chromosome at the exact location or to stay in the form of an episome within the nucleus (it should not be installed randomly in the chromosomes of the host, since it would mean a disturbed control of expression). The existing vector systems can be briefly divided into viral and non-viral. The paper presents the basic principles of the application of genes in the therapy of atherosclerosis, hereditary lung disease and cancer. *Acta Medica Medianae 2011;50(3):74-80.*

Key words: gene therapy, genes, expression, atherosclerosis, hereditary lung disease

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Introduction

Individual differences in pharmacological response of patients to administered drugs lead to serious clinical problems, of which the most important are difficult treatments, adverse drug reactions and interactions among drugs. There is a growing body of evidence that the observed characteristics in pharmacokinetics and pharmaco-dynamics of drugs are genetically determined and that they primarily include drugs metabolism and transport processes. Current acknowledgements, based on collected evidence, clearly show that an early adjustment of therapy regime to genetic characteristics of patients may help to avoid side effects. Such an approach stands for an individualized, optimal therapeutic use of drugs, based on the dose regime adjusted to an individual genotype. Differences in patients` response to a specific drug therapy may be explained by genetically conditioned differences in a drug metabolism, its distribution in the body and target proteins through which a drug achieves its effects. In recent years, a special attention has been drawn to a connection between the effects of drugs and genetic polymorphism of transport proteins, then, to the target molecules through which drugs achieve their effects, as well as receptors, enzymes and proteins involved in intracellular signal transduction.

Gene therapy

Gene therapy is the intentional transfer of genetic material into human somatic cells in prophylactic, therapeutic or diagnostic purposes. While the gene transfer technology is very advanced, ethical principles related to this procedure are still the subject of discussion. The goal of gene therapy is to correct genetic defects and to establish normal cell functioning. Most of the techniques of gene therapy should enable the replacement of defective genes by those that function normally. Exogenous genes, called trans-genic, can be transferred into somatic (organs) or germ-line (egg cells, sperm) cells of a recipient.

Gene transfer to somatic cells means that genetic changes are not passed on to offspring. Conversely, gene transfer into germ-line cells (which is illegal in many countries including the

USA.) results in genetic alterations of newborns (1). Gene therapy of diseases is one of the most important achievements of medicine, which offers great opportunities of treating both inherited and acquired diseases. The target changes in cells, tissues and organs, prolonged effects and the improved therapeutic index are achieved by this therapy. Most importantly, gene therapy removes the cause of the disease. Outstanding achievements in molecular medicine and genetics of the last decade have enabled the clinical application of the gene transfer technology. Initially, the focus of gene therapy was on hereditary diseases such as cystic fibrosis, severe immunodeficiency, hemophilia, sickle cell anemia, adenosine deaminase deficiency. Gene therapy researches were later expanded to acquired diseases, primary malignant tumors and heart disease.

Although the first study with patients began in 1990, a clear success was achieved only a few years ago (2-4). Nowadays, over 600 clinical studies of gene therapy are conducted, most of which (63%) are related to cancer therapy.

Gene transfer systems

The biggest obstacle in the development of gene therapy as an effective clinical method was finding a suitable vector that would transfer genetic material into a target tissue. Although a considerable progress has been achieved and several gene transfer systems developed, this problem remains a limiting step in the application of gene therapy to a wider range of diseases.

Desirable characteristics of an ideal vector are:
1. To be able to pass big transgenes;
2. To enable transduction in vivo of active as well as cells at rest;
3. To have an effect on the target tissue with minimal local damage;
4. To be immunologically inert;
5. To integrate into the genome of a cell or persist in the cell as an episome within the nucleus;
6. To ensure a continuous expression;
7. To be a subject of post-transduction regulation;
8. To be produced in large quantities for commercial purposes (5).

The ideal vector should influence specific target cells, without stimulating the inflammatory response in the host cell. In addition, it should facilitate the transfer of genes of different length (the length of the code gene therapeutic sequences vary considerably and there should be a possibility of inserting regulatory sequences in transduction and expression) and integrate into the host cell chromosome at the exact location or to stay in the form of an episome within the nucleus (it should not be installed randomly in the chromosomes of the host, since it would mean a disturbed control of expression) (6). The conditions for a widespread commercial production should also exist. A vector with these properties has not been produced yet.

Existing vector systems may be briefly listed as:
1. Viral (e.g., adenoviruses, retroviruses)
2. Non- viral (e.g., exposed DNA, liposome).

The advantages of using non-viral vectors are the following: reduced immunogenicity, a more extensive production, ease of use and unlimited size of genes that can be transferred, and the disadvantages: an inadequate gene transfer and transient expression (7, 8).

Viral vectors are currently the most efficient carriers of genes, enabling an extended gene expression, but are highly immunogenic. There is a limit to the size of genetic material that can be transferred (because of the size of viral capsid) and it is difficult to ensure a safe production of a larger number of these vectors (9, 10). Up to now, the application of gene therapy in the treatment of non-cardiovascular diseases has relied primarily on the use of viral vectors, whereas, non-viral vectors have been used in the studies conducted on cardio-vascular diseases, primarily exposed DNA or liposomes.

Viral vectors

Viruses are intracellular parasites, usually with a specific affinity to a particular type of cell. The life cycle of viruses consists of two distinct phases: infection and replication. During infection, the viral genome enters the cell and soon after a larger quantity of viral regulatory peptides is created. After a while, structural proteins are also synthesized, which results in the creation of new viral particles. Viral vectors used for gene therapy have an altered genome and contain therapeutic genes, rather than, for example, sequences necessary for viral replication. In other words, active viral regulatory sequences attached to therapeutic genes are introduced into a cell and a viral particle, unable to replicate, but able to transfer the desired genetic material into the target cell is created (10).

Each viral vector is characterized by special features that make it suitable for the treatment of certain diseases. For example, so that the gene therapy for stopping the growth of tumor cells by introducing a suppressor gene would be successful, it is necessary to transfer genes into a large number of abnormal cells (11). Another strategy involves the application of the genes carrying information for protein synthesis, which can perform the conversion of antitumor pro-medicines into toxic chemicals. A successful treatment in this case will be enabled by introducing genes into a smaller number of cells (12,13). The third approach in tumor therapy involves the application of oncolytic viruses, genetically altered viruses that can replicate only in tumor cells. The result is lysis of cells (14).

All viral vectors can be divided into two groups, depending on whether the genes they carry do or do not integrate into the genome of host cells. Retroviral vectors (including lentiviruses) belong to the first group, while Adenoviral vectors remain in

the form of the episome within the host cell nucleus. Of all the viral vectors up to now, retroviruses have been used the most (38%), followed by adeno-viruses (25%), but some new vectors are increasingly being used such as herpes and pox viruses (15). Non-replicable viral vectors (viral vectors that are not selective for cells that are replicated) are retroviruses, adenoviruses, herpes simplex virus, pox virus, vaccinia virus, baculovirus and others.

Non-viral systems

Non-viral systems were developed to avoid some security problems related to the use of viral vectors. They are easier to use, easier to manufacture and do not generate a specific immune response (16). However, previous researches have not provided a satisfactory clinical success, so the research continues. Non-viral systems are:

1. Exposed DNA (plasmids): In 1990, it was found out that exogenous genes can be expressed after direct injection into a muscle. That, for example, led to the development of DNA vaccines that stimulate a cytotoxic T-lymphocyte response.

Previous findings indicate that a gene transfer in vivo with long-term expression in some tissues is effective, but also that it is weak in the target tissues.

2. Cationic lipids: are the synthetic genes obtained from DNA complex / lipids (17). They provide a good ex vivo transfection and were used for getting CFTR (transmembrane receptor in cystic fibrosis) gene that was brought in through the nose and lungs of patients with cystic fibrosis (18). However, gene expression was not long enough for a clinical success. Progress in this area is small.

3. Molecular conjugates (condensed DNA particles) are being developed (19).

Gene therapy was originally considered in the treatment of monogenic diseases, such as cystic fibrosis. In this case, the mutated gene should be replaced by a healthy one. However, that involves finding the vector that would perform the gene replacement in a large number of cells and either permanently or for a longer period of time. This kind of vector has not been produced yet. The application of gene therapy in treating cardiovascular diseases includes the recognition of disorders in which transient gene expression may be sufficient to correct the disorder. For example, the expression of genes throughout 2-3 weeks may be all that is necessary to accelerate neovascularization or prevent restenosis. In addition, by using genes which encode a protein and are normally secreted in the body, their paracrine effect can be used to overcome the need for the introduction of viral vectors in a large number of cells.

In the past few years a considerable progress has been made in the production and processing of viral vectors, so that it no longer poses a major obstacle in the selection of vectors for a clinical study. The vectors mentioned in the paper are being improved and it is very likely that the discovery of new agents with improved characteristics will

continue. The regulation of gene expression is particularly important after its entry into the cell via a vector. This would allow the activation of transgenes when necessary; maintaining their expression in exactly defined time and ending. It is not possible to expect that a vector can be applied to all aspects of gene therapy, taking into account the characteristics it should have. The modern concept of application of vectors is the following: use of adenoviruses in gene therapy of tumors by entering genes in charge of oncolysis; exposed DNA vacci-nation and the delivery of genes for angiogenesis in the treatment of cardiovascular disorders, application of AAV genes for the therapy of chronic diseases such as chemophylia (8, 17, 19).

Gene therapy for atherosclerosis

Genetic interventions for atherosclerosis can be carried out: directly (in vivo) or indirectly (ex vivo).

The direct gene transfer includes methods of inhibition or amplification of expression of target (target) genes through a direct application of viruses, lipids, oligonucleotides and recombinant DNA. High hopes are placed in the study of vascular remodeling and angioneogenesis by changing the expression of growth factor by gene transfer. Gene therapy can lead to stabilization of an activated, complicated plaque and inhibition of the secondary creation of thrombus in the wall. Reduction of macrophage infiltration and inhibition of the so-called foam cells should reduce the risk of rupture and hemorrhage by the dissection of vulnerable plaque. Considering the fact that nitric oxide (NO) inhibits the chemotaxic activity of monocytes and their accumulation in the intimae during atherogenesis, transfer of genes responsible for transcription of NO synthesis could lead to the stabilization of plaque (20). Given that the recruitment and infiltration of monocytes in atherosclerotic plaque include the essential role of protein-1, which attracts monocytes (MCP-1), a new antiMCP-1 strategy of gene therapy has been developed, by the transfection of a mutated gene that has a deletion (7ND) at N-terminal end in skeletal muscles. This transfer results in a decrease in infiltration / activation of monocytes after the injury of arteries and inhibition of restenosis with experimental animals after the balloon dilatation and stent incorporation (21).

The indirect (ex vivo) method involves extraction of cells that play a role in key metabolic activities, their genetic modification and re-implantation of altered cells that are able to create a new protein and / or enzyme material or to alter the expression of specific genes. The concept of this method is extended to prevent late complications such as hyperplasia of new intimae after arterial reconstruction (endarterectomy, bypass, stent insertion) (22, 23). Featured endothelial cells of patients are genetically modified in vitro. Injection of these cells enables the coverage (endothelial seeding) of arterial grafts and/or stent by genetically modified endothelial cells. In familial hypercholesterolemia, the genes responsible for

synthesis of enzymes that activate LDL receptors are added to isolated liver cells, after which they are brought back to patients` liver. In this way, with the same patients, more risk factors (hypertension, hypercholesterolemia, intimae hyperplasia, can be eliminated at several levels by genetic interventions (24).

Gene therapy of inherited lung diseases

The two most common hereditary lung diseases in Europe are cystic fibrosis and alpha1 antitrypsin deficiency. Cystic fibrosis is an auto-somal, recessive disease caused by a mutation in the CFTR gene (Cystic Fibrosis Transmembrane Conductance Regulator) located on the long arm of the 7th chromosome. The most common mutation (70%) is the deletion of three-base-pair. CFTR gene modulates the secretion of protein C1 (transmembrane channel) in response to the elevation of intracellular adenosine mono-phosphate (AMP). Mutations of this gene lead to the loss of function in epithelial cells, which leads to the increased divergence of transepithelial potential. This gene is localized in epithelial cells of the respiratory tract, pancreas, intestines and sweat channels. The disease is clinically manifested primarily in the lungs, gastrointestinal tract and pancreas. A simplified explanation of the origin of respiratory disorders is that CFTR gene defect prevents adequate hydration of fluid in epithelial cells, which leads to mucus, obstruction and subsequent infection and inflammation. Given that in over 90% of cases, death is caused due to respiratory disorders, gene therapy of this disease is focused on the genetic defect of lungs. CFTR gene expression is very low, and only about 10% of the cells should be adjusted in order to stabilize a patient (25).

Alpha1 antitrypsin deficiency is an autosomal disease in which hepatocytes do not secrete sufficient amounts of this` protein in serum, although a certain amount is synthesized in monocytes (macrophages). It results in the accumulation of alpha1 antitrypsin in hepatocytes, but low serum levels. The lungs are most affected by this deficiency due to the loss of antiprotease protection, while it leads to the manifestation of toxic effects due to accumulation of alpha1 antitrypsin in the liver. The most significant clinical manifestation is panacinar pulmonary emphysema, which occurs in younger children, covering and destroying predominantly the lower areas of lungs. Gene therapy of inherited lung diseases includes the ex vivo and in vivo strategy. The ex vivo strategy of gene transfer into the lung parenchyma has not been satisfactory for the following reasons:

1. It is very difficult to grow the epithelial cells of the respiratory system in a cell culture;

2. Retroviruses, which are mostly used as vectors, require cell division, while the epithelial cells of the respiratory tract are well differentiated;

3. Lung surface is about 140 m^2 with 23 generations of bronchi;

4. Given that the alpha1 antitrypsin is produced in the liver, the target organ in this case should be the liver itself.

Most of the research carried out in the in vivo strategy involves using gene transfer by adenoviral vectors and plasmid-liposis complex, either intra-venously or by aerosol. Adenoviral vectors have the advantage because of the efficiency of gene transfer, and the disadvantage is their relative toxicity or induction of immune responses and symptoms that are similar to a cold (26). Plasmid-liposis complex has the advantage of its easy production and low toxicity, but the low efficiency of gene transfer is its disadvantage. The latest studies have shown the advantages of adeno-associated viral vectors and hybrid vectors of plasmid-viruses, for which the advantages of both vectors should be used (27).

Gene therapy of cancer

Contrary to the gene therapy of inherited nonmalignant diseases when it is enough in many cases to genetically change only a small number of cells to achieve the remission of the disease, gene therapy of cancer must draw each malignantly transformed cell in order to reach a complete eradication of cancer. Specifically, with hereditary diseases that occur due to insufficient function of a gene and a lack of protein encoded by the gene, establishing of gene function in a number of cells will lead to coding and creating the missing protein that establishes the balance. With such diseases, the missing protein functions by the principle of "sufficiency", that is, small amounts of this protein are sufficient and able to govern the given disorder. There is no negative effect in case of over-expression of the gene and the occurrence of large amounts of encoded protein than sufficient one. On the other hand, if gene therapy does not destroy all cancer cells, the remaining cells continue to multiply and cancer continues to evolve. Cancer gene therapy should discontinue the function of oncogenes or restitute the function of tumor suppressor genes, that is, the defective gene should be replaced by a normal gene by the process called homologous recombination. Unfortunately, the efficiency and reliability of this process is very low. For now, by the in vitro and in vivo it is only possible to insert genes into a cell, but not to substitute it, i.e. a defective gene remains in its place. For these reasons, the current cancer gene therapy is moving in four directions:

1. Transfer of a suicide gene that converts an inactive nontoxic drug into the cytotoxic agent;

2. Transfer of genes that encode certain cytokines and stimulating factors to increase the immune response against the tumor;

3. Transfer of tumor-suppressor genes in order to block tumor cell proliferation and,

4. T ransfer of «drug resistance» genes in haematopoetic stem cells in order to increase the resistance to myelosuppressive chemotherapeutic drugs (27, 28).

Tumor suppressor gene therapy

Loss or inactivation of certain genes may be an important event in the pathogenesis of cancer. These genes suppress cell proliferation and are called tumor suppressor genes, anti-oncogenes or recessive oncogenes, given that there is a mutation or deletion of both alleles in transformed cells.

Products of oncogenes are effects of transformation. They activate the cell to the expression of a transformed phenotype and are considered as positive regulators of growth. Products of tumor suppressor genes are negative regulators of growth and the loss of their function leads to the expression of the transformed phenotype.

One of the tumor suppressor gene is p53, which is frequently mutated in lung cancer cells. It is a nuclear phosphoprotein encoded by genes located on the short arm of the 17th chromosome. It is one of the most frequently altered genes in human malignomas and it clearly has a key role in regulating normal cell growth. The normal product of p53 gene (wild-type p53) has a function in the regulation of DNA transcription and cell transformation. The loss or mutation of this gene leads to abnormal gene expression and consequently to unregulated cell growth, because the mutant p53 has no ability of suppressing cell transformation. Wild type p53 has a role in apoptosis of cells with damaged genetic material that cannot be repaired. If there is a chance of repairing damaged genetic material, wild-type p53 retains the cell in G1 phase, allowing genetic repair, and only then "allows" the cell to go to the next S phase (DNA transcription) and subsequent phases of cell division. If the genetic material in G1 phase cannot be repaired, it leads to apoptosis. In this way, wild-type p53 protects cells from transformation. Mutated p53 does not have this ability, so the cell with damaged genetic material is not retained in the G1 phase, and there is no chance of reparation. It is not a subject to apoptosis and it enters with such altered genetic material in the subsequent phases of cell division, which consecutively leads to malignant alteration of cells. It is extremely important that the wild-type p53 is able to inhibit cell growth regardless of the genetic abnormalities that led or will lead to a malignant expression. This ability is used as the base for wild-type p53 in gene therapy of cancer. Given the role, the wild-type p53 has proven to be very convenient and potent for gene therapy. Its transfer is performed by using recombined retroviral vectors and adenoviral vectors. The basic difficulty in this gene therapy is the insufficient number of transductional cancer cells necessary for a complete recovery. Moreover, the bystander effect plays a positive role in this case. Clinical researches have beliefs in the routine application of a tumor suppressor gene therapy in vivo. Tumor suppressor gene therapy gives remarkable results in clinical studies on the local treatment of lung cancer (adenoviral vector is administered directly into the tumor by bronchoscopy) in an advanced stage. Therapeutic possibilities of a tumor suppressor gene therapy with p53 gene are in combination with radiotherapy and chemotherapy in the systemic application by using liposomes (28).

Conclusion

Similar to some other new therapeutic concepts, gene therapy is promising, but for now it is still being developed. The main challenge is bringing the right gene into the right place and the right cell and providing an adequate expression, with minimal side effects. Although most work is done on viral vectors, it is considered that the future of gene therapy is based on much safer non-viral systems. There have been some failures in gene therapy, which led to suspicion and concern in the general population. However, the development of gene therapy is our reality, as well as the fact that it has its place in medicine. It is important to emphasize that gene therapy should be approached with an extremely high degree of scientific, professional and ethical responsibility, because the possibility of genetic manipulations dangerous to human health cannot be excluded.

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MOGUĆNOSTI I DOMETI TERAPIJE GENIMA

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Sve je veći broj dokaza da su zapaženi induvidualizmi u farmakokinetici i farmakodinamici lekova genetski determinisani i da primarno obuhvataju metabolizam lekova i transportne procese. Dosadašnja saznanja, bazirana na sakupljenim dokazima, jasno pokazuju da rano prilagođavanje terapijskog režima genetskim osobinama bolesnika može pomoći da se izbegnu neželjena dejstva. Ovakav pristup znači individualizovanu, optimalnu terapijsku primenu lekova baziranu na doznom režimu koji je prilagođen individualnom genotipu. Genetska terapija predstavlja nameran prenos genetskog materijala u humane somatske ćelije u profilaktičke, terapijske ili dijagnostičke svrhe. Dok je tehnologija za transfer gena vrlo usavršena, etički principi vezani za ovaj postupak i dalje su predmet brojnih rasprava. Cilj genetske terapije je da koriguje genetske defekte i da uspostavi normalnu ćelijsku funkciju. Većina tehnika genetske terapije treba da omogući zamenu defektnih gena onima koji normalno funkcionišu. Idealni vektor bi trebalo da deluje na specifične ciljne ćelije, bez stimulacije inflamatornog odgovora domaćina. Osim toga, trebalo bi da omogući prenos gena različite dužine (dužine kodirajuće sekvencije terapijskih gena se znatno razlikuju i trebalo bi da postoji mogućnost ubacivanja regulatornih sekvencija za transdukciju i ekspresiju) i da se integriše u hromozom ćelije domaćina na tačno određeno mesto ili da ostane u vidu epizoma unutar nukleusa (ne sme se dopustiti da se ugradi nasumice u hromozome domaćina, jer bi to značilo poremećenu kontrolu ekspresije). Postojeći vektorski sistemi mogu se ukratko podeliti na virusne i nevirusne. U radu će biti izloženi osnovni principi primene gena u terapiji ateroskleroze, naslednih plućnih bolesti i malignih oboljenja. *Acta Medica Medianae 2011;50(3):74-80.*

Ključne reči*: genetska terapija, geni, ekspresija, ateroskleroza, nasledne plućne bolesti*