Gene Therapy and Coronary Artery Disease

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Key words:
Gene therapy, genetic vectors, coronary artery disease, myocardial infarction.

Manuscript received:
August 23, 2000;
Accepted:
December 11, 2001

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The whole human genome consists of 3 million pairs of bases that encode almost 50.000-100.000 functional genes. It is forecasted that within the next decade, the whole human genome will be decoded, revealing the secrets of the human genetic code[1]. In the light of this achievement, the possibility of therapeutic intervention at genome level becomes more and more evident, aiming at preventing and treating a plethora of diseases. The term "gene therapy" has thus been established, for several years now, indicating several therapeutic techniques that have been developed towards this direction. As gene therapy we consider the introduction of some form of nucleic acid in the cells of the patient in order to improve the course of a disease or to prevent its manifestation[1]. Usually, the nucleic acid involves part of the double DNA helix (gene) that encodes proteinosynthesis.

In accordance with the current gene therapy techniques, there are three alternatives in the application of gene therapy[1,2]:

a) Replacement of ineffective genes for the treatment of hereditary diseases.

b) Enhancement of the normal gene activity or introduction of additional gene information affecting the course of diseases associated with cell proliferation.

c) Restriction of the expressiveness of genes contributing to the pathogenesis or maintenance of the pathogenesis mechanism (e.g. oncogenes).

Principles of gene therapy application

Gene therapy today is applied experimentally via two large categories of techniques: in vivo and ex vivo, as shown in figure 1[1].

1. In vivo and ex vivo gene therapy[1-4]

This distinction of several protocols of gene therapy is based on whether the transfer of the gene takes part inside the human body (in vivo) or outside the human body (ex vivo).

In accordance with the ex vivo technique, allogenic or autologous cells that are to be "inoculated" with the said genetic material are removed from the body, they are "contaminated" by the respective genetic vector and are re-introduced in the patient's body[4]. Naturally, the ex vivo technique requires the possibility to preserve cell cultures, something that renders it difficult to use. Another prerequisite of these techniques is also the ability of proliferation and preservation in culture of the cells to be "inoculated" with the new genetic material. Furthermore, the removal and re-insertion of the tissue to be modified requires major surgical interventions. It is precisely due to these disadvantages of the ex vivo gene therapy that this method is used today mainly in cells of the hematopoietic system, the skin, the vascular endothelium as well as in neoplasm cells.

In the case of in vivo gene therapy, the genetic material is directly introduced to the body, in such a way so as for it to be able to reach the target-tissue, where it will penetrate the cells of this tissue and will start being expressed in them. This methodology can be applied when the target-cells cannot easily develop out-

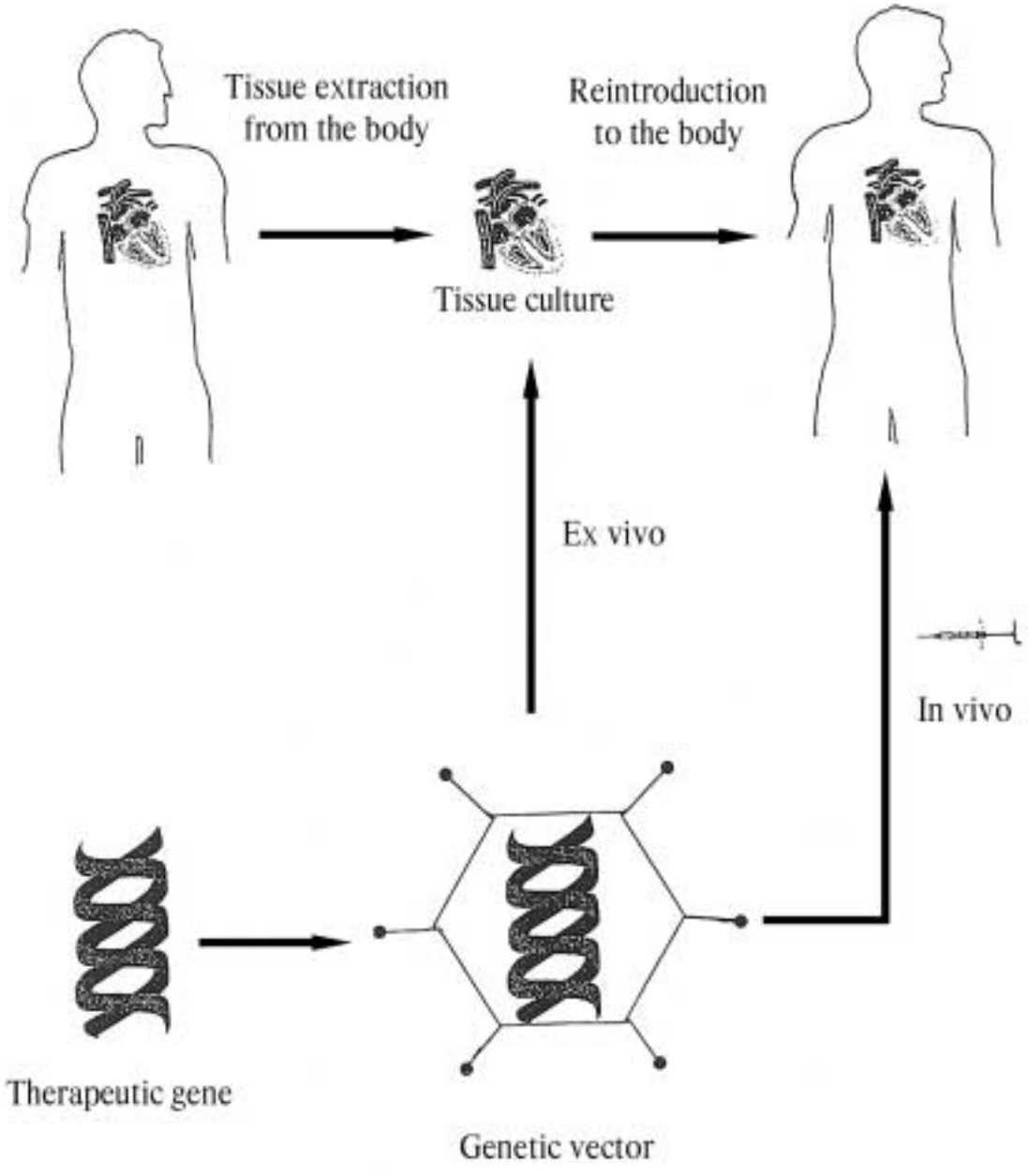

Figure 1. Basic principles of gene therapy. After the insertion of the therapeutic gene into a genetic vector, the gene is transferred to the body with the in vivo or the ex vivo technique, as described in the text.

side the human body. Naturally, the *in vivo* introduction of genetic material in the body, although particularly attractive, is still at a very early stage.

2. Somatic and germ cells gene therapy

Gene therapy is also divided in two main categories, depending on the type of cells to which the genetic material will be added:

(a) Germ cells gene therapy is the type of gene therapy according to which the genetic material is inserted in germ cells, thus being transferred to the descendants.

(b) Somatic gene therapy is the type of gene therapy according to which the genetic material is inserted in somatic cells. This is the type mainly used in cardiology and more specifically in the treatment of coronary heart disease[1].

Table 1.

Viral vectors	Non viral vectors
1. Retroviruses	1. Cationic liposomes
2. Adenoviruses	2. HVJ liposomes
3. Adeno-Associated Virus (AAV)	3. «Naked» DNA
4. Other viruses	

Genetic vectors in gene therapy

In order for the genetic material to be inserted in the target-cells, either with the in vivo or with the ex vivo technique, certain vectors are required where the genetic material is initially inserted. These vectors then serve as "transport vehicles" of the genetic material to the cells. The vectors that have been occasionally used are listed in table 1[1].

1. Viral vectors of genetic material in gene therapy[1-6]

Viral vectors have the advantage of exploiting the natural mechanisms of the body's cell receptors, thus increasing their efficacy. Moreover, they lead to a stable and long-term expression of the genetic material in the cells where they have been inserted. However, their use is limited due to reservations regarding both their safety as well as their immunogenicity.

a. Retroviral vectors of genetic material

Retroviruses are the most commonly used viral vectors in the different experimental protocols throughout the world. More specifically, retroviruses are currently used in several research protocols representing 46% of the vectors used, followed by liposomes (19%) and adenoviruses (18%). The remaining vectors are used much less.

Retroviral vectors of genetic material were developed in the '80s and constituted the first vectors ever used in gene therapy, in cardiology and particularly in vascular diseases. Researchers' interest in these vectors was increased when they observed that they efficiently attack cultured cells, in which their genetic material remains and is expressed for large periods of time in vivo, following the insertion of such cells in the body.

The retrovirus is bound to a specific membrane receptor of the target-cell and penetrates in its interior through membrane fusion. In the cytoplasm, the viral RNA, through the effect of the reverse transcriptase viral enzyme, leads to the production of the respective DNA. The viral DNA is later incorporated in a chromosome of the host-cell.

In order to use retroviruses in gene therapy, we should first render them incapable of proliferating within the cell. Thus, in order for such a virus to be used as genetic material vector, the gag, env and pol genes must first be cleared from its genome, since they encode respectively the production of the nucleic protein, of the viral envelope protein as well as of the reverse transcriptase. To replace such genes in the viral RNA, we introduce nucleotides that correspond to the gene that we wish to incorporate in our target cells[1]. The proteins that the modified viruses lack (gag, poly and env products) are provided to them through the insertion of the modified retroviral plasmid in cell lines that express these products (packaging cells). In this way, the retroviruses that are unable to proliferate are collected from the supernatant of this packaging cell line and are used for the genomic transfer of the gene they contain.

These viral vectors have a capacity of transferring genomic material up to 7-10 kb[1].

Retroviral vectors also possess the capacity to attack a large variety of cells, introducing to them the genetic material they carry. However, a prerequisite for their action is the active proliferation of the target-cells[5]. More specifically, the retroviral vectors are used in ex vivo gene therapy. In accordance with this technique, the cells to undergo genetic modification are removed from the body and initially are cultured under controlled conditions. The retroviral vector is then injected in the culture, bringing to the cultured cells the gene to be inserted[6].

After the "inoculation" of the cultured cells with the new genetic material, such cells are re-inserted in the body, where the encoding of the gene begins, to the benefit of the recipient[6].

Retroviral vectors cannot be used in the in vivo technique. This is due both to the fact that retroviruses are neutralized in the body by the complement, as well as to the fact that the cells we wish to be attacked by the virus are not necessarily in the proliferation phase. For this reason, retroviral vectors constitute an excellent means of gene transfer only with the ex vivo technique. Their use is reported, for example, in the treatment of vascular disease, where cells of the vascular endothelium are attacked in culture by the respective retrovirus and they are

later re-inserted in the vascular wall, as will be analyzed later on.

The advantages of the use of retroviruses are[1,2,3]:

a) They provide consistent results lasting for months.

b) They do not induce local inflammatory reactions.

c) They have no immunogenic properties.

The disadvantages of the use of retroviruses are[1,5]:

a) They cause mutations in the cells genome due to the incorporation of unknown DNA.

b) They have very limited efficacy in vivo and their use is restricted to in vitro and ex vivo techniques.

c) They only attack proliferating cells.

b. Adenoviruses as vectors of genetic material[2]

After the adenovirus, in its natural form, binds with a membrane receptor in the surface of the cell to be attacked, it penetrates the cell through endocytosis. The viral DNA is then introduced in the nucleus of the cell, starting to express its genes. The expression of the viral genes takes place both with the participation of cellular transcription factors, as well as with the expression of E1 region of the viral genome. So, the depletion of E1 region of the viral DNA, using molecular biology methods (homologous recombination method) and its replacement with foreign cDNA, renders the adenovirus incapable of proliferation. Thus, the newly formed adenovirus carries the gene we want to insert to the target-cells without damaging them. Of course, it preserves its potential to infect new cells without, however, damaging them[3]. Up to 10kb of genetic material can be transferred in this way.

The advantages of the use of adenoviruses as vectors of genetic material in gene therapy are[1-4]:

a) A characteristic of the adenoviral genetic vectors is the non-incorporation of the genetic material that they carry in the cellular genome, that renders them particularly safe, since the risk of mutation due to introduction of foreign genes in the cellular genome is avoided. The gene that enters the cell in this way, remains in the nucleus in the form of episome and does not enter a cellular chromosome.

b) The non-incorporation of the foreign DNA in the cellular genome is the cause of a very short duration of action of this form of gene therapy (only a few weeks). This, on the one hand, constitutes a disadvantage for the treatment of chronic diseases and, on the other hand, an advantage for the treatment of conditions such as restenosis following angioplasty, where short duration of action is mandatory.

c) They may be used in vivo and not necessarily ex vivo, as is the case with the retroviral vectors.

d) Unlike retroviral vectors, they have the ability to also affect non-proliferating cells.

The disadvantages presented by the adenoviral vectors used in gene therapy are also significant, while their possible solution in the future could eventually pave the path for the development of an extremely efficient gene therapy method. These disadvantages include[2,3,5]:

a) Reduced expression in vivo due to the immunological response (humoral and cellular) that the adenoviruses induce to the host.

b) Induction of significant local inflammatory reaction in the tissues that they affect.

c) As it has already been mentioned, they present a limited duration of action (currently 2-3 weeks).

c. Other viral systems for the transfer of genetic material in gene therapy

Several other viruses have been used experimentally for the transfer of genetic material within the framework of gene therapy. Among them are the following: Adeno-Associated Virus (AAV), Herpes Simplex Virus (HSV), pertussis virus, cytomegalovirus (CMV). In particular, the cytomegalovirus has been detected in atherosclerotic plaques, a fact that makes it an eligible vector for the treatment of atherosclerosis (as for example in the prevention of restenosis following angioplasty).

2. Non-viral genetic vectors in gene therapy

In the last few years, several non-viral methods for the transfer of genetic material have been used, both *in vivo* as well as *in vitro*, within different experimental protocols of gene therapy. Up to date, however, most non-viral genetic vectors have not been used in clinical studies, mainly because of their inability to present a controlled and satisfactory expression of the genes that they carry in special target-tissues. Such genetic vectors may carry any volume of genetic material to the target-cells without any restriction, since they use plasmids for its transfer[1,2].

The potential for effective insertion of naked DNA in a target-tissue, following intravenous administration, is very low. The use of plasmid DNA

has been applied only in the case of direct needle injection (puncture) in specific muscular tissues without particularly good results[7]. This is exactly why vectors such as cationic liposome and HVJ liposomes have been used, since they form stable complexes with plasmid DNA and they transfer it to the specific tissues that will be injected with it.

Liposomes as genetic vectors in gene therapy[1,2,5]

The cationic liposomes have been used as vectors in gene therapy thanks to their capacity of transferring genes inside the cells in *in vitro* cultures. Their use in vivo, however, is counter-indicated due to the instability presented by the liposome - plasmid DNA complex in blood. This instability is due both to the action of the complement on the complex as well as to its rapid clearance, through the action of the reticulo-endothelial system.

The non selective "attack" of tissues by cationic liposomes seems to have been partially resolved with the use of liposomes as genetic vectors, that carry on their surface some substances attached, recognized by special receptors of specific cell populations in the body.

The selective introduction of liposomes in the target-cells is thus achieved through endocytosis, following the mediation of the respective cellular receptor. Asialoglycoprotein, transferin etc. have been used as such surface molecules. The main problem of this method is that the receptor - surface molecule complex is not only directed to the cell nucleus where the transferred DNA must be installed in order for it to be expressed. To the contrary, this complex initially settles in a newly formed endosome in cytoplasm, where it is degraded if it does not succeed to escape quickly from it.

The efficacy of liposome vectors may also improve with the use of the heat-inactivated HVJ virus (hemagglutinating virus of Japan) in the formation of HVJ-liposome gene vectors.

Consequently, despite the fact that non-viral genetic vectors seem quite attractive due to their ease of production and their property not to induce immunological response in the body, their efficacy in gene therapy remains to be proven. Aside from their inefficacy in vivo, the small period during which the transferred DNA can be expressed inside target-cells (approximately 1 month), renders them even more deficient genetic vectors[2].

Applications of gene therapy in coronary artery disease

Gene therapy has been applied (at an experimental level) in many branches of medical therapeutics and naturally in cardiology. Genetic intervention on the several parameters of the coronary heart disease currently constitutes the focus of international research. More specifically, important progress has been made in the application of gene therapy for conditions such as atherosclerotic disease, thrombosis, restenosis following angioplasty, myocardial infarction, hypertension and transplant graft rejection (Table 2). The discovery of a genetic basis in a plethora of pa-

Table 2.

Gene therapy in coronary artery disease: applications

Target component	Inserted gene
Atherosclerosis	
• familial hypercholesterolemia	LDL-receptor
• common hypercholesterolemia	Antioxidants, adhesion molecules, NO-synthase, etc.
Thrombosis	hirudin, tPA, etc
Restenosis following angioplasty	PDGF and FGF inhibitors, etc, NO-synthase, HSV-tk, cytosine deaminase
Following myocardial infraction and ischemia- reperfusion injury	MyoD, Ec-SOD, VEGF, etc
Hypertension	Angiotensin II receptor antagonists, b-blockers, kallistatin, etc
Transplant grafts rejection	b-galactosidase, IL-10, p40 subunit of IL-12, etc

thological conditions has provided the opportunity of genetic intervention in many of these conditions. As it is obvious, the intervention in monogenic diseases (whose manifestation is due to the presence in the genome of one dominant or two allelic recessive genes) is an easy target for gene therapy. Familial hypercholesterolemia is reported as such hereditary disease[4,7]. Of course, another target of gene therapy is also the intervention in multigenic diseases such as atherosclerosis and many others.

1. Gene therapy and atherosclerosis

In the last few years, a plethora of articles have been published on the applications of gene therapy in atheromatic disease[7,8]. In the application, however, of gene therapy in atherosclerosis, we must take into consideration the causative distinction of the disease in monogenic forms (such as familial hypercholesterolemia) and multigenic forms, that affect most of patients suffering from it.

a. Monogenic atherosclerotic disease: familial hypercholesterolemia

Familial hypercholesterolemia is due to a specific gene mutation of the gene that encodes LDL receptor[4,7]. The genetic intervention in this hereditary disease has been sought both through the *ex vivo* as well as the in vivo gene therapy technique.

According to the ex vivo technique, hepatocytes of a patient with homozygous familial hypercholesterolemia are surgically removed and are cultured *in vitro* together with a retrovirus that carries the normal gene for the production of the LDL receptor. The tissue is then re-implanted to the patient, thus leading to 20% reduction of LDL levels in plasma and reduction of the LDL:HDL ratio from 10-13, before the application of the therapy, to 5-8[4].

On the other hand, the application of the in vivo gene therapy in familial hypercholesterolemia has also made satisfactory progress. More specifically, adenoviruses are prepared with the use of molecular genetics techniques, carrying the healthy gene and they are later introduced in the body. The Rous Sarcoma Virus (RSV), containing the gene for the LDL receptor, has been used experimentally in animal studies. After the virus attacked the hepatocytes of the animals, it led to the expression of the gene in them. As a result, LDL levels in plasma were reduced by 75%, attaining a minimum level within 6 days from the application of one dose of the virus. LDL returned to baseline three weeks later[4].

b. Multigenic form of hypercholesterolemia

When the genetic vectors used in gene therapy will overcome the several problems that exist in their application, the clinical treatment of multigenic atherosclerosis will turn towards two directions: a) Towards a systemic level, where the several clinical protocols will focus on the increase of lipids metabolism and b) Towards a local level, where research will focus on the insertion of genetic material through a catheter (directly or with the use of a vector) to the wall of the artery to be treated[7,8]. This genetic material includes genes encoding different adhesion molecules[7,8], anti-oxidative factors[9], or molecules related with the local nitric oxide production (NO) such as NO-synthase[2,3,10]. An alternative approach in the treatment of atheromatic lesions in the coronary arteries involves the introduction of genes producing molecules that induce the development of collateral circulation at the sites of stenoses[7].

2. Gene therapy and thrombosis

Arterial thrombosis that is caused by the rupture of atheromatic plaque in the coronary arteries, plays an important part in the manifestation of myocardial infarction.

In studies, the use of a recombinant adenovirus containing a gene for the production of hirudin, induced thrombin inhibition in a study model of carotid lesions in mice[7]. Also, the transfer of the gene responsible for the tissue plasminogen activator (tPA), through an adenoviral system, in animals with a deficient fibrinolytic mechanism, led to significant increase of the fibrinolytic activity and prevention of thrombosis[7].

Thus, there could be room for the clinical use of systemic administration of a genetic vector, aside from its local administration (through a catheter), in order for patients with a predisposition to arterial thrombosis to benefit from the enhancement of the anticoagulation systems of their body.

3. Gene therapy and restenosis following angioplasty

The pathophysiology of restenosis following angioplasty includes, as is already known, elements common to atherosclerosis as well as to thrombosis,

and, therefore, in many points, gene therapy for such conditions is common[7].

As we know, restenosis presents a particularly complex pathophysiology. This involves: proliferation of smooth muscle cells of the vascular wall, accumulation and adherence of platelets, formation of thrombi, activation of inflammatory cells, vascular remodeling, etc. All these elements in the pathophysiology of the disease may constitute different points of intervention of the gene therapy[3]. The application of gene therapy at the level of migration of smooth muscle fibers is currently the best studied strategy.

In many recent studies, it has been found that the local administration of growth factors (such as PDGF and FGF) at the point of vascular lesions significantly increases the proliferation and migration of smooth muscle cells. Thus, within the framework of gene therapy, the transfer and local expression at the site of angioplasty of a gene preventing the expression of PDGF and FGF factors (e.g. of a gene producing altered molecules of such growth factors, so that they can bind to the respective receptors, without stimulating those intracellular activities that would normally be activated by the active growth factors) has been used. Naturally, the overlapping of the several intracellular pathways with which the action of growth factors is exerted, reduces the efficacy of gene therapy, when this turns against the action of a small number of such factors[3]. Thus, research today focuses on the application of the genetic intervention in "key" sites, that constitute a junction between the different intracellular pathways[3].

The migration of smooth muscle fibers seems to be partially regulated by the local levels of endothelial nitric oxide[3,11]. Thus, the over-expression of nitric oxide synthase at sites of vascular lesions inhibits to a satisfactory extent the proliferation of smooth muscle cells[3,12].

The production of nitric oxide has of course the additional advantage of inhibiting platelets accumulation, while it has a vasodilating action[3].

Another type of gene therapy that has been applied with success in the last few years for the treatment of restenosis involves the introduction of a gene responsible for the conversion of a non-toxic drug to toxic, in the area where this gene is expressed. An example of this strategy is the insertion of a gene encoding thymidine kinase of the Herpes Simplex Virus (HSV-tk gene) at the site of the vascular lesion, with the use of an adenoviral vector. The cells expressing the HSV-tk gene, when exposed to gagcyclovir, can phosphorylate it and convert it to a nucleotide analogue that, incorporated in the cellular DNA, irreversibly blocks its duplication. Experiments in animal models, towards this direction, have had impressive results with regard to the prevention of restenosis following balloon angioplasty, since there was an inhibition of smooth muscle cells proliferation at the vascular wall[13].

Another example of gene therapy similar to the previous one is the introduction of a gene expressing the production of cytosine deaminase, bacterial enzyme. This enzyme may metabolize 5-fluorocytosine, an approved, relatively non-toxic antifungal drug, to 5-fluoro-uracil, an antimitotic chemotherapeutic agent. This conversion leads to selective cytotoxic action against cells expressing the above gene, that is against migrating smooth muscle cells[14].

The advantage of this strategy, i.e. of the introduction of genes producing non-toxic products that can convert a non-toxic agent to toxic, is that the toxic action of the treatment may be immediately stopped, when necessary, with the cessation of administration of the drug to the patient[3]. Of course, the use of the said gene cannot be selective to the smooth muscle cells and as a result, endothelial cells are also destroyed, which leads to some other adverse events of this therapeutic approach. The development of adenoviral genetic vectors with increased cytotropism towards specific cells (such as vascular smooth muscle cells) may prove to be a solution to this problem in the future[3,7].

4. Gene therapy following myocardial infarction and ischemia-reperfusion

As was previously mentioned, gene therapies that have been designed against the development of atherosclerosis or arterial thrombosis, can also play a protective part against the manifestation of myocardial infarction[7]. However, gene therapy can also be applied in the post-myocardial infarction period. In the last few years, some efforts have been made to prepare appropriate genetic factors (such as adenoviral genetic vector ad5), able to attack myocardial cells following a myocardial infarction[7]. Consequently, two strategies have been developed for the re-creation of functional muscle cells at the site of manifestation of the infarction and the repair of the non functional myocardium.

In accordance with the first strategy, the genetic re-programming of cardiac fibroblasts in the area of the infarct has been attempted in order for the fibroblasts to manifest a muscular phenotype[7,15]. Towards this direction, researchers have tried the introduction of the gene for MyoD (myogenic commitment factor), in order to promote the post-infarct differentiation of myocardial fibroblasts to striated muscular tissue[15].

A second strategy involves the effort to produce functional myocardium following the introduction in the cardiac myocytes of the appropriate genetic information, in order for them to proliferate, colonizing the neighboring infracted areas of the myocardium[7,16].

Both such approaches involve the application of gene therapy on the heart tissue, after it has suffered the infarct lesions. If, however, they are applied preventively, they can, theoretically, protect the myocardium from the lesions caused by the ischemia-reperfusion procedure in heart-attacks[7]. Such an approach could be clinically tested on specific high-risk populations, after safe and efficient methods of genetic material transfer are developed.[7] For example, during the first 2 weeks following a myocardial infarction, the average mortality rate ranges from 2% to 4% and this is due, among others, to the possibility of manifestation of a new myocardial infarction[7]. In this case, gene therapy could be applied for preventive purposes.

"Anti-oxidative" gene therapy has been used experimentally, in the last few years, against the natural consequences of the ischemia-reperfusion lesion, following myocardial infarction[17].

The intravenous administration of an adenoviral vector, carrying the gene for the extracellular isomorphous form of EC-SOD, leads to the re-programming of hepatocytes and to a significant increase of the levels of this anti-oxidative enzyme in blood (this is achieved through the addition of hirudin, thus achieving the removal of the enzyme from the extracellular receptors where it is bound and its entrance in systemic circulation)[17]. The above experiment indicated that mice that had been subject to this genetic treatment, presented significant resistance against myocardial infarction[17]. Of course, other clinical or laboratory studies are also required to prove the efficacy of this genetic intervention in the prevention of myocardial lesions during reperfusion following myocardial infarction.

In the last few years, clinical studies have applied the insertion of the VEGF (growth factor) gene in the myocardium cells, in order to stimulate the collateral circulation procedure in patients with heart ischemia. This has been attempted through the direct injection of plasmid ("naked") DNA in the myocardial cells with satisfactory results[18,19,20].

5. Gene therapy and hypertension

Gene therapeutics has tried in the last few years to intervene towards the direction of regulating arterial hypertension as well as its consequences to the heart. The ability of the angiotensin converting enzyme inhibitors and of the inhibitors of angiotensin II (type I) receptors to reduce arterial pressure and to reverse left ventricular hypertrophy, renders the renin-angiotensin-aldosterone system (RAAS), a particularly attractive target for gene therapy. Specialized vectors of genetic material could, for example, be used in order to program hepatocytes in such a way so as to be able to produce angiotensin II receptor antagonists or b-blockers[7].

The correlation of RAAS and of the kallikrein-kinine system, has been exploited in the genetic control of hypertension. The ad5 adenoviral genetic vector has been experimentally used to transfer the kallistatin gene in hypertensive mice[21]. Kallistatin is an inhibitor of tissue kallikrein that after binding with it, it inactivates it. The use of ad5 vector and the transfer of the said gene led to arterial pressure reduction in hypertensive mice for more that 4 weeks[21].

6. Gene therapy and graft rejection

The area of transplant surgery constitutes an exceptional field of gene therapy application. This is due to the fact that an ex vivo genetic transfer system can act on the tissue to be transplanted, in order to introduce genes in it, in an absolutely controlled way, without the risk of systemic diffusion in the body. Thus, the insertion of genes to the graft, presenting long-term expression that will have a protective action against the mechanisms of graft rejection by the host, may prolong its viability[22].

Many experimental studies have been conducted towards this direction, showing encouraging results. These studies have attempted to prolong the time from heart transplant to the activation of the mechanisms of its rejection. Genes expressing molecules such as b-lactosidase[23], viral interleukin 10 (IL10)[24,25],

p40 subunits of IL-1226 and many other genes have been used from time to time. The production of such graft protecting molecules from cells of the graft itself, has been found to prolong its stay in the host organism, providing hope for the solution of the immunorejection problem in heart transplants, following future clinical studies.

Conclusions

The application of gene therapy has already started to exceed the narrow boundaries of experimental investigation, at the level of laboratories and a large variety of clinical protocols has been approved and are currently under way. Despite the practical and ethical dilemmas it gives rise to, application of gene therapy in humans has begun. Naturally, certain rules have been established regarding the procedure to be followed within these clinical trials, whose application is deemed necessary[27]. The primary aim of these rules is, among others, the protection of the genetic cells of patients subject to gene therapy, without which the human species could be led to destructive paths with no return.

Despite all that, after gene therapy surpasses the problems it faces in its application, it is expected to revolutionize therapeutics and in general all the fields of medicine in the decades to come. Therefore, the huge problem of coronary heart disease may also find its solution in the future through the paths of gene therapy.

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