ABSTRACT

Background: The subject of this paper is gene doping, which should be understood as “the non-therapeutic use of cells, genes, genetic elements, or of the modulation of gene expression, having the capacity to improve athletic performance”. The authors of this work, based on the review of literature and previous research, make an attempt at wider characterization of gene doping and the discussion of related potential threats.

Methods: This is a comprehensive survey of literature on the latest applications of molecular biology in medicine. The analysis involves a dozen scientific databases examined in order to find genes used in gene therapy and potentially useful in gene doping.

Results: The obtained results enable better recognition of gene doping and indicate genes used in medicine that could be used in gene doping. This paper describes potential effects of their use and associated risk, and predicts the possible developments of gene doping in the future.

Conclusion: Gene doping is undoubtedly a part of modern sport. Although WADA included gene doping on the list of banned methods as early as 2004, as previously stated above, it has not managed to develop efficient methods of detection.

KEY WORDS: gene doping, sport, doping, gene therapy.
INTRODUCTION

One of the most promising directions in research aiming at increasing the functional capabilities of the human organism is connected with the latest achievements in molecular biology. However, the use of the achievements of modern genetics for sport purposes, in spite of potentially unlimited possibilities, is ethically ambiguous and is associated with many often unpredictable threats. The most obvious example is 'gene doping' which should be understood as “the non-therapeutic use of cells, genes, genetic elements, or of the modulation of gene expression, having the capacity to improve athletic performance”.

A publication in Sport Medicine from 2004 (15) quoted the chairman of WADA (World Anti-Doping Agency), prof. Friedman, as saying the improvements in gene therapy would make gene doping inevitable in sport within the following several years. In parallel predictions of other WADA officials, the first cases of illegal gene doping would have already been used during the Olympics in Beijing in 2008 (16).

The widely publicised case of the coach of the German national team, who tried to buy Repoxygen (used in gene therapy to stimulate the production of erythropoietin), implicates that practices of this kind took place earlier, during the winter-Olympics in 2006 (11). It is impossible to confirm this 100%, because as yet, in spite of several years of research, no efficient detection methods have been found for gene doping.

So what exactly is gene doping? Can it (regardless of the ethical dimension of the problem) really bring success in sports and what are the associated threats?

METHODS

This survey is an analysis of literature on the latest applications of molecular biology in medicine. The analysis involves a dozen scientific databases, examined in order to find genes used in gene therapy and potentially useful in gene doping. The gathered data are supplemented and verified by conversations with leading Polish university clinics that include gene therapy in their treatment methods.

RESULTS

The best starting point for the explanation of gene doping is a review of gene therapy, a technique of correcting damaged genes, or even supplementing their inherent deficiency, through the introduction of suitable DNA
fragments into the genetic structure of a given organism. Gene therapy is used to save human health and life, but the aim of gene doping is perfecting and improving the body of a sportsperson, increasing their capability of breaking barriers and improving sport achievements (2).

The basic carrier of genetic information is deoxyribonucleic acid (DNA). All the cells of the human body a DNA chain with about 30 thousands genes (DNA regions responsible for coding a specific types of protein, eg. enzymes important for life processes). The essence of gene therapy is the introduction of therapeutic genes to cells, whose task is to compensate for damaged genes or substitute the missing ones. In effect, one can 'shift' the cell to produce a given type of protein, or to the contrary-inhibit the expression of certain genes.

Nowadays, the preparation of a desirable region of DNA in laboratory conditions is not a very difficult task. The simplest method is extracting the suitable coding region and 'gluing' it with a new and more active region. Then the prepared DNA fragment is introduced back into the body (most often using vectors, eg. viruses without genes responsible for their life cycle). This introduced genetic material becomes a matrix for mRNA, formed during transcription. The subsequent process, ribosome translation, is the final translation of genetic information from the DNA code into the specific protein structure (Figure 1).

![Figure 1: The general principle of gene therapy (1. Pathogenic genes are removed from the genetic material of the virus and substituted with a therapeutic DNA; 2. The patient is infected with the genetically altered virus; 3. The therapeutic viruses reach impaired cells, where the transferred gene is read. The cells resume their normal function.)](image)

The practical application of gene therapy in medicine became possible thanks to the unusual development in molecular techniques at the end of the 20th century. One of first patients on which gene therapy was used was Ashanthi DeSilva, suffering from ADA-SCID. The therapy started in 1990 - previously isolated lymphocytes were given a healthy copy of the damaged gene, and then the lymphocytes were re-introduced into the body of the girl. An-
other early case of gene therapy was an attempt at using it on patients suffering from talasemia. Researchers from California University in Los Angeles collected cells from the marrow of two patients, introduced a correct gene of haemoglobin and grafted the marrow back into the body. Unfortunately, the therapy gave no positive result.

Even more unfortunate was the case of 18 year old Jesse Gelsinger. His organism did not cope with the decomposition of unused amino acids, which consequently caused attacks of coma, amyotrophy and arduous vomiting. In 1999, he was one of eighteen volunteers who registered at the Institute of Gene Therapy at the University of Pennsylvania, for clinical research on their diseases. Unfortunately, within 96 hours of the use of gene therapy, Jesse Gelsinger died as a result of the strong immunological reactions of his body.

In spite of the fact that gene therapy is still at the stage of experimental research and that only in selected clinical cases is it used on people, its potential is immense (2). It is estimated that the development of this method will enable an efficient treatment of 6000 previously incurable diseases (11). In sport, the method will certainly help in the treatment of some types of traumas and contusions. Experimental research proved that the introduction of genes of suitable growth factors directly into the site of a trauma (eg. IGH-1 whether EGF) considerably accelerated the regeneration of joint cartilages, copuli and tendons (9). Unfortunately, as previously stated, the use of gene therapy in sport probably will not be limited only to permissible forms of treatment.

Gene doping the non-therapeutic use of gene therapy, having the capacity to improve athletic performance. Literature on the subject does not report any particular genes used in gene doping (it is still non-detectable and nobody would admit to using it). However, there is a whole range of means used in gene therapy that could increase the chance of success in sport competition. In most cases their effects have only been examined on animals but some have already been applied in medicine. It is important, however, to be aware that all the genes taken into account here are only ‘candidate genes’.

Below we present genetic factors that are most frequently mentioned in specialist literature in the context of their non-therapeutic application in doping.

**DISCUSSION**

One of the potential examples of the prohibited use of gene therapy in sport is the administration of the erythropoietin-coding gene, erythropoietin being a stimulant of different stages of erythropoiesis. A high concentration of this hormone directly corresponds to the quantity of red blood corpuscles, in-
creasing blood supply and muscle oxygenation. Sportspeople who use EPO doping take this hormone in the form of injections up to a dozen or so times a week. After the cessation of treatment (e.g. the specimen Neorecormon), the level of hematocrit relatively quickly comes back to normal levels, and additionally, this form of doping is relatively easily detectable. In the case of the gene doping, DNA given only once may maintain an elevated level of hematocrit for a very long time. Moreover, such doping is practically undetectable.

The beginnings of EPO gene therapy date as early as 1994. In an experiment on mice, EPO activating gene was injected, the level of hematocrit increased from 48% to 70% and remained at that level for 4 months (14).

The level of hematocrit (normally about 40%), after a single injection of a virus containing EPO coding gene in two baboons, increased rapidly as early as in the 4th week of the experiment and remained on 60-70% level for several months (18).

Another example of gene therapy potential like gene doping is the application of VEGF gene which is responsible for coding of growth factor of vascular endothelium. This factor is responsible for the process of angiogenesis, i.e. the formation of new blood-vessels. In medical care, VEGF gene therapy is used in ischemia disease of heart (5) and in ischemia diseases of lower limbs (1). The example results of gene therapy activating the gene VEGF are presented in the photos below (Fig. 2).

Figure 2: The increase in the number of blood-vessels as a result of VEGF gene therapy. Left drawing presents the state of blood-vessels in the patient with the ischemia disease; Right drawing presents the state of blood-vessels in the same patient after an injection activating the VEGF gene.

In the case of patients suffering from ischemia diseases, the aforementioned gene therapy gives a chance of avoiding the amputation of the affected limb. The use of the same method in fully healthy sportspeople (as gene doping) will cause a similar effect - an increase in the the quantity of blood-vessels. In this way, the blood supply will increase, along with the oxygena-
tion of muscles and organs, which will significantly increase their resistance to fatigue. Potential advantages are self-evident; a decrease in the threshold of fatigue and an improvement in the reaction of the body to performed physical effort, which are basic goals of training, not only for athletes and swimmers, but in most sport disciplines.

Gene therapy may potentially be used in body building and power sports. Here, the most frequently reported examples of potential gene doping refer to insulinopodobnogo growth factor 1 (IGF-1) and myostatin.

The administration of genetic material IGF-1 during an experiment on young mice caused an increase in muscular mass and speed by about 15-30%. An increase was also observed in the number of nuclei in myocytes (13).

Similar results were achieved in experiments deactivating the gene of myostatin - the negative growth regulator of muscles. In the effect of applied genetic modification (on mice), "Schwarzenegger mice" were obtained, characterized by a greater power and faster increase of muscles than control mice (7). An example of blocking the myostatin gene is presented in the photo below (Fig. 3).

Figure 3: The effect of blocking the myostatin gene. Left drawing shows the limb of a normal mouse, and right drawing shows the limb of a mouse with a blocked myostatin gene.

The aforementioned potential possibilities of gene doping are of course only examples concerning only several out of dozens of genes. In 2006 a significant correlation to sporting achievements was confirmed for 170 genes, and the number continues to grow (1).

As previously stated, gene doping, similar to gene therapy, is in the early phase of experimentation. Nevertheless, modern research on the development of gene doping shows several different directions that may be developed in the future.
Presently used methods of gene therapy enable the introduction of genes into the body (most often in the form of episomes, i.e. plasmid DNA fragments which can integrate with the chromosome of the host). Although no method has been found for substituting individual genes in human cells with other genes, some researchers predict the development of such a method in the nearest future (6). It would enable the exchange of some forms of genes for improved versions, and finally a permanent change in individual features of the body.

Another important issue and a subject of intense research, is the possibility of using a transcription factor in gene therapy. Modern methods are based on single genes coding specific types of proteins, e.g. hormones. Such methods have many limitations associated for example with the range of their influence (8). Modern research will in the future help increase the efficiency of gene therapy based on activating transcription factors, i.e. specific proteins found in each cell of the body whose task is to control the activity of each group of genes (3). This method would enable activation or blocking of gene sets (not single genes, as presently). Potentially it would enable a change in the whole phenotype of man.

It must be stated, however, that the realization of this direction of research is still a matter for the future. Current possibilities of applying gene therapy in treatment are much smaller and, as previously stated, such methods are usually in the experimental stage (although several thousand people worldwide have received such treatment). It does not mean, however, that they will not be used in sport, even in the face of the huge risk associated with their use.

The greatest threat connected with gene doping, which probably is the most evocative, is the potential possibility of cancer. A DNA fragment, after introduction into the human body, can cause a change in the genome, which consequently can have fatal results. One should mention here the gene therapy used in severe combined immunodeficiency associated with X (SCID-X). Among 11 boys subjected to the therapy, 3 developed leukemia (15). The possibility of developing cancer as a result of VEGF gene therapy was also reported by Haisma (5).

Another potential threat associated with gene doping (and gene therapy too), results from the incapability of controlling gene expression due to the limited possibility of influencing the site in the genome where the gene is introduced. Looking at a report by Schjerlinga (12) which described the introduction of the EPO coding gene where it caused a permanent increase in the production of erythropoietin in experimental monkeys, which resulted in such a high level of hematocrit that it started to pose a direct threat to life. Still worse, the process could not be stopped in any way. In the future, the problem might be solved by the use of regulatory promoters, but the success of such treatment will always demand the constant monitoring of patients.
A very strange, and still not fully explained, phenomenon is a process opposite to the aforementioned excess gene expression. In a study analogous to the aforementioned one, the introduction of EPO coding gene caused a severe ischemia occurred in experimental monkeys (4).

The reason for this situation was the autoimmunologic response of the body, although the detailed mechanisms of this response are still not recognized completely. The possibility of a similar response in humans was mentioned by Unal (15).

A risk factor that is most frequent in gene therapy, is an immune response by the body to the vector used for gene introduction. Most often vectors are viruses with the pathogen removed, and their introduction to the human body results in a natural response by the immune system. In extreme cases (as in Jesse Gersinger’s case) this reaction can cause severe organ dysfunction that may even lead to death.

One of the greatest problems in applying gene doping is the lack of efficient methods that could stop any undesirable effects. Unlike the case of incorrectly applied medicines which can be stopped and the activity of which can be neutralized, the side effects of gene therapy cannot be stopped. Is it possible then that there are sportspeople who in spite of the potential threats still decide to use gene doping?

CONCLUSION

Research by the American Olympic Committee (17) showed that about 70% of the surveyed sportspeople would have had a surgical sex change operation if it had guaranteed an Olympic medal. If there are such people who are so determined to achieve their professional goals, it seems they would also not hesitate to use gene doping. But can it be counteracted?

Among a whole range of genes (in 2008 there were 180 of them) with a confirmed effect on sport performance, only some can be used in gene doping. The effect of this part has been confirmed in studies on animals, although some of them have also given good results in treating people.

Despite the increasingly common use of gene therapy in medicine, the use of gene doping is still associated with a tremendous risk. On the other hand, this type of doping is still undetectable and much more effective than the 'traditional' doping.

The main problem in estimating the risk in using gene doping is the very small number of reports on this subject matter. In most cases (and also in this paper), only theoretical possibilities are considered when it comes to the use of specific genes and their potential effects, as nobody would ever admit to using gene doping. On the other hand, nobody denies the very existence of gene doping. It is only the scale of this type of doping which remains unknown.
Although WADA included gene doping on the list of banned methods as early as 2004, as previously stated above, it has not managed to develop efficient methods of detection. Several scientific centers work on the efficient methods of fighting against gene doping, but it is completely unclear if it will ever be possible. Presently used solutions require muscle biopsy (for which nobody will ever agree), or are based on indirect methods (restrictions concerning the sales of gene therapy preparations). Unfortunately, the efficiency of these methods is controversial.

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