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POTENTIAL THREAT IN FUTURE

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Abstract: Definition of gene doping by World Anti Doping Agency (WADA) is as "the non–therapeutic use of genes or cells that have the capacity to enhance athletic performance". Due to recent research related to genome and particularly the success of Human Genome Project, robust data is available about genes that influence performance and endurance in athletes. Gene doping is an unwanted brainchild of gene therapy. Gene therapy is used for medical treatment but gene doping is intended to change the function of normal cells in a healthy sports person. The transfer of gene could be performed by following methods: i) by injecting the gene directly into the muscle, ii) inserting genetically modified cells into the body, iii) using a vector to deliver the gene. Concerns are raised about the possible use of gene doping in sports in near future. In accordance with this threat, possible detection methods have also been developed. According to WADA, the expression of the inserted gene could be measured as a change in a particular protein or enzyme, or an increase in production of red blood cells.

Keywords:Gene, doping, gene transfer.

Introduction:

In spite of the strict measures by International authorities doping has always been a very tempting option for many athletes which would possibly bring laurels to them. Athletes will go to any extent where winning is considered as of supreme importance. There is a prominent fear in sports community that apart fron drug doping, a new form of doping will emerg in near future which could be scarcely preventable and not detectable. This new and more potent, more vicious form of doping is known as gene doping or genetic doping. Definition of gene doping by World Anti Doping Agency (WADA) is as "the non–therapeutic use of genes or cells that have the capacity to enhance athletic performance". The use of these genes are able to increase the strength of muscles, have the power of regeneration and even prevent them from degradation. DNA resides in the nucleus of the cell and is the basic carrier of genetic information. This information is expressed in the form of thousands of types of proteins and enzymes which are extremely important for life processes.

Gene therapy is a technique of correcting damaged or mutated genes, through the introduction of suitable or correct or normal gene into the genetic structure of a given organism. Gene therapy is used for life saving purposes, for improving the disease condition, for compensating the damaged gene or for replacing the missing gene but the aim of gene doping is intended to change the function of normal cells in a healthy sports person, perfecting and improving the body, increasing their athletic ability of breaking barriers and improving sport achievements. Due to recent research related to genome and particularly the success of Human Genome Project, robust data is available about genes that influence performance and endurance in athletes. Gene doping is an unwanted brainchild of gene therapy.

Gene Doping

Gene doping, which could be explained as "the non-therapeutic use of cells, genes, genetic elements, or of the modulation of gene expression, having the capacity to improve athletic performance" (Pawel et al, 2009) The WADA (World Anti-Doping Agency) defines gene doping as the "non-therapeutic use of genes, genetic elements and/or cells that have the capacity to enhance athletic performance".

What makes gene doping so attractive and according to the athletes what are the advantages of gene doping over drug doping? Primarily, the most advantageous feature of gene doping is that it is not detectable in blood. Changes in the gene sequence will result in the synthesis of a chosen protein which is very same to its original gene which makes the detection very difficult. An additional beneficial point for athletes is use of high muscle force required in various events and skeletal muscle is an important target for gene therapy. Due to certain properties of skeletal muscles like its large size, non-proliferative nature of the tissue, the product of the gene remains for longer time in the tissue.

Advances in gene therapy could one day make it possible for any athlete to enhance their DNA. For example, in experiments aimed at treating muscular dystrophy in the elderly, a group led by physiologist Lee Sweeney of the University of Pennsylvania in Philadelphia introduced a gene to cause over-expression of IGF1 in mice. The treatment boosted muscle strength of young adult mice by 14%, earning the rodents the nickname 'mighty mice'. (Thompson H.,2012). Injuries derived from sports practice is a major factor of early drop out from the sports career, longer time away from training and competitions, as well as downgradation in performance. (Patel DR and Baker RJ, 2006).

Also, important tissues involved in the good athletic career like tendons, ligaments and cartilage are very difficult to regenerate. (Huard J, et al, 2006). The gene therapy could, therefore, have a very important application in the sports field, including the reconstitution of injured tissues. Nevertheless, this kind of treatment always carry a potential threat of misuse by athletes who search for physical performance improvement. The misuse of this therapy is called gene doping and has been issue of a scientific-academic debate whose importance has been growing in sports medicine and sports sciences(Haisma HJ, et al, 2006). Gene therapist Ted Friedmann and multiple Olympic gold medallist Johann-Olav Koss were the first to describe the possibility of misusing the techniques and experiences of gene therapy in the athletic arena. In 2006, before the Turin Winter Olympic games, the president of the World Anti-Doping Agency (WADA), Dick Pound, called gene doping "the new threat that is now a reality." Although Pound did not expect gene doping to pose a problem in Turin, he indicated that it could be a problem at the Summer Games, 2 years hence in Beijing. In fact, the problem did not materialize in China, in 2008, nor at the London 2012 Olympics, as far as the then available detection measures could determine . (ÅkeAndrén-Sandberg, 2006). The ability to give athletes the "best" version of each gene for their chosen sport could improve performance. Even more significantly, if we could manipulate genes easily it would be possible even to create versions of genes not found naturally, giving athletes supra-physiological amounts of key gene products. Effectively, we could create superhuman athletes. (Colin Moran, 2016).

Direct injection into the muscles of athletes would be simple and very difficult to detect . As gene doping becomes more efficient, it is likely to offer great opportunities for doping in sport (Andersen, Schjerling&Saltin, 2000). Detection will likely require not blood or urine tests, but invasive, difficult and dangerous muscle biopsies. As gene therapy works in animals nowadays, there is no reason why it could not be attempted by athletes. (Foddy B. And Savulescu J., 2007)

However , there are many potential risk factors involved. Clearly, gene doping would amount to cheating, making for an uneven and unfair means of winning. But the most obvious risk is that it doesn't work. While viruses have spent millions of years evolving ways to get into our cells, we equally have been evolving ways to stop them. Our immune systems can react against the modified viruses used to "implant" new or altered genes, rejecting them totally. Indeed, poorly executed gene therapy could make us sick like any other viral infection or worse. There is also the potential for off-target effects, whereby the gene therapy process unintentionally alters some of our healthy genes, creating unexpected side effects. Even if gene doping were to work, changes

that help athletes improve their performance may not prove to be good for their long-term health. The infamous drug EPO, used by cyclist Lance Armstrong, increases the red blood cell count, allowing blood to carry more oxygen. This is good for aerobic exercise. However, those extra red blood cells also make blood thicker, leading to an increased risk of stroke. (Colin Moran, 2016). Virulent viral gene therapy vectors may be produced which puts forth a major safety concern. In the case of virulent viruses, these are not only harmful to the athlete, but also pose a health risk for the general population who might get infected. Health risks resulting from expressed genes are similar to those of other doping forms. However, the level and duration of protein production is difficult to control when compared to conventional protein administration. For example Epo delivered by gene therapy could result in sustained high Epo levels which would increase the chances of stroke and heart attack. (Haisma H. J. et al 2004).

Since the DNA can get integrated into the genome, the risk of cancer is always present. Integration will alter the gene that the construct by chance happens to be integrated into. (Schjerling P., 2005). Cancer is due to unfortunate alterations of genes so the possibility of cancer cannot be ruled out. In fact, the two recent cases of cancer caused by gene therapy treatment of immunodeficiency were most likely due to integration into a latent cancer gene (Hacein-Bey-Abina et al., 2003).

Methods:

Gene doping is 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 best possible options (Pawel C., 2009). The process of introducing genes into cells is known as gene transfer. In spite of the common notions to the contrary, in many cases the gene change thus induced is impermanent, being a transitory modification that needs repeated therapy. The gene of interest could be inserted either into somatic cells or into germ cells . In Germany only somatic gene therapy is allowed. There is a broad international accord that germ-line therapy is scientifically and ethically unacceptable at present, due to the incalculable risk of transmission and diffusion of the transferred gene in the human population. Depending on the gene-transfer method used, somatic gene therapy is divided into in vivo and ex vivo techniques. In ex vivo therapy, cells are removed from the body. The corrective gene is then inserted into those cells in a laboratory, after which the cells are put back into the body. Ideally, the cells migrate to their site of action, where they multiply, differentiate, and start producing the missing protein. However, only a few types of somatic cells can be cultivated outside the body (e.g. blood cells), and few of these can be successfully reintroduced into the body.

In in vivo therapy, the therapeutic gene is inserted directly into cells inside the body. This gene-transfer approach would be desirable for reasons of efficiency, but it is encompassed by a number of practical problems. The vectors injected into the blood are rapidly diluted. En route through the body they encounter many cell types that are unaffected by the disorder concerned. The insertion of therapeutic gene is through transfer vectors which are able to recognize the target cells. There are many insertion systems of the genetic material in vivo. The commonest vehicles are the viral vectors ,including the most widely used retrovirus and adenovirus. The genetic information of these viruses is inserted into the chromosomes of the recipient cells and is passed on when the cells divide. In principle this allows the efficient production of gene therapy proteins but can also cause severe side effects, including cancer, due to integration in nuclear DNA. The DNA of the

adenoviruses remain outside the chromosomes rather than being integrated within them. This limits the possible duration of action but also prevents the adverse effects of integration. However, severe immune reactions remain a problem. Another category is adeno-associated viruses. These particularly small, harmless viruses usually integrate at a specific site within nuclear DNA without severe consequences and with a very high expression rate of the transferred genes. Their major disadvantage is their limited capacity to transport genes.

So far mostly viral vectors have been used in gene therapy. Viral vectors are viruses that are unable to replicate and cause potential disease. They have been genetically modified so that they are harmless and so that they are able to transport normal DNA into cell nuclei. Recently a growing use of nonviral vectors has been observed. There is no such thing as the ideal vector; each must be adapted specifically to the nature of the genetic defect being treated (form of administration, tissue specificity, expression characteristics, etc.). The challenge of gene therapy lies in designing the best vector for treating the disease concerned. Before being introduced in the patient, the virus used as vectors is treated i.e. changes are made which makes them safer for use, such as several genes which give it virulence are removed or inactivated (Wilson DR., 2004, Reifenberg K, et al, 2006, Rubanyi GM. 2001).When incorporated in the person's the target cells, the viral vectors inject their genetic material containing the therapeutic gene in the person's DNA, enabling the synthesis of its corresponding functional protein, or they use the molecular equipment of the host cell to express its genes. Haisma and Hon (Haisma HJ, de Hon O. 2006) affirm that around 3000 patients have received some kind of gene therapy. Several diseases have been treated, including endothelial dysfunctions, hemophilia, immune deficiency and many kinds of cancer (Rajagopalan S, et al, 2003, Losordo DW, et al, 2002, Patel DR, et al, 2006, Hacein-Bey-Abina S, et al, 2002, Kay MA, et al, 2000).

Other approaches for inserting the gene includes the use of naked DNA. The integration of naked DNA in somatic cells (without the biological insertion mechanisms of viruses) is greatly limited but can be enhanced by lipofection (coupling to suitable molecules) or electroporation (use of electrical pulses). (KatrinGerlinger et al, 2009). However, several other types of non-viral vectors have also been used, such as liposomes and macromolecules conjugated to the DNA (Wilson DR, 2002, Reifenberg K, et al, 2006, Rubanyi GM. 2001).

The injection of genetic material straight to the target tissue is also a way of performing the gene therapy without the use of virus (. Wilson DR, 2002, Reifenberg K, et al,2006, Rubanyi GM. 2001). There is also the gene therapy system ex vivo , in which the cells of the patient himself are removed , altered and reimplanted in the patient, so that the therapeutic gene is inserted outside the patient's organism (Karthikeyan BV, and Pradeep AR., 2006).

Despite the scientific and technological advances, there are still many doubts concerning the side effects of the gene therapy. The introduction of genetically modified organisms generates a great uncertainty, especially if the virus mutagenic potential is considered (Unal M, and Unal DO, 2004). Nevertheless, there is no doubt that the main problem the gene therapy faces in the current stage of development is the high immunogenic capacity of the viral vectors and the fear of severe immune reaction, which can be a major complication. (Tan PH, 2006, Ritter T, et al, 2002, Bangari DS, and Mittal SK. 2006). Although non-viral vectors are an interesting treatment alternative, problems associated with their use are efficiency, toxicity and inflammatory response . (Li S-D, and Huang L. 2006). Despite being developed with the purpose to treat severe diseases, gene therapy, as well as several other therapeutic interventions, has great potential of abuse among healthy athletes who wish to improve performance. (GuilhermeGiannini et al, 2007).

Myostatin

Myostatin is a growth factor which controls muscle growth. One family has been identified with a genetic mutation resulting in no myostatin production (Schuelke, et al., 2004). This particular mutation is found to be beneficial for the affected child resulting in extraordinarily strong and developed muscles. Similar results have been obtained in genetically altered mice which do not produce myostatin have huge muscles and have been called Schwarzenegger mice (Lee, 2004). Injecting the mice with myostatin blockers cause significant increase in muscle mass (Lee &McPherron, 2001). Genetic manipulation to stop myostatin production or administration of blockers would be expected to significantly increase strength in athletes and are likely to offer real potential for doping in the future. Insulin-like growth factor injected into the muscles of mice increases strength. (Foddy B., and Savulescu J., 2007). Myostatin is a negative regulator of muscle formation. Synthesized by muscle cells it acts either auto- or paracrine in heart and skeletal muscle. Its physiological role is still not yet clear. Administration of myostatin blockers such as follistatin, mutant activin type receptors and myostatinpropeptide, will result in a dramatic and widespread increase in skeletal muscle mass due to an increase in number of muscle fibres (hyperplasia) and thickness of fibres (hypertrophy) and less fat and connective tissue in muscle (Lee and McPherron, 2001). These myostatin antagonists may improve muscle regeneration in patients suffering from Duchenne and Becker muscular dystrophy (Bogdanovich et al., 2002).

Gene doping strategies would thus aim to inhibit production of myostatin or interfere with the function of the endogenous protein. (Harridge S. And Vellosi C., 2008).

Erythropoietin (Epo)

At the Olympic Games in Innsbrück, Austria,Finnish Nordic skier EeroMäntyranta blew away the competition and won two gold medals. It was later shown that Mäntyranta had a naturally occurring genetic mutation that gave him higher amounts of red blood cells than the average person. Having more red blood cells means more cells to carry oxygen from the lungs to tissues, thus increasing his stamina. Athletes of the future may be able to alter their genes in a way that mimics the natural mutation that Mäntyranta had. This may be accomplished by inserting an additional copy of a gene into a person to boost production of the hormone erythropoietin (Epo). This hormone instructs the body to synthesize new red blood cells. For athletes, increased Epo production would enhance oxygenation of tissues, in turn increasing endurance. Epo may be delivered as a protein by injection, or by introduction of the gene encoding Epo into the body's cells. Researchers successfully delivered Epo genes into the cells of mice and monkeys .(Zhou et al.,1998). Although promising, pain relieving gene therapy is still in its infancy and far from clinical application. (Gene doping , Haisma H. J., et al, 2004).

Insulin-like growth factor-I (IGF-I)

Like gene therapy for Epo production, techniques to strengthen muscles are being developed to help people with illnesses: in this case, people with degenerative muscle conditions such as muscular dystrophy. Whereas the Epo therapy would be pervasive throughout the body, this approach would target specific muscles. Insulin-like growth factor-I is synthesized in the liver as well as muscle and has anabolic effects. Its concentration is related to the concentration of growth hormone GH. IGF-I gives rise to an increase in muscle bulk in mice injected with the gene (Barton-Davis et al., 1998). This was in the absence of any special exercise programme. Extending this treatment to athletes could mean strengthening the precise muscles. Such gene therapy is likely to be relatively safe given that the effects seem to be localized to the targeted muscle and is likely that human trials will start in the coming years.. However, before clinical studies can be started, further studies in primates need to be performed to further evaluate the efficacy and toxicity of IGF-I for gene therapy. (Haisma H. J., et al, 2004).

IGF-1 is a 70-amino-acid polypeptide synthesized primarily in the liver under the control of GH. The GH/IGF-1 axis is extremely important in regulating postnatal growth and development. In addition to the liver, other tissues, including skeletal muscle, can produce IGF-1. The IGF-1 gene comprises six exons and a process of alternative splicing at the 5and 3ends can generate different isoforms. Evidence from viral gene transfer studies in mice have shown that the two murine 3splice variants IGF-1Ea and IGF-1Eb (also termed mechano-growth factor or MGF) can induce significant local muscle. (Harridge S. And Vellosi C., 2008).

Vascular endothelial growth factor (VEGF)

Genes may also be used to help grow new blood vessels. This therapy is being developed to produce a coronary bypass in patients with ischaemic heart disease and may help elderly people with peripheral arterial disease, which is the death of tissues in the body's extremities because of inadequate oxygen supply. The gene encoding vascular endothelial growth factor (VEGF) or other factors stimulate synthesis of new vessels. The clinical trials in many instances show efficacy in patients with angina (Losordo et al., 1998; Losordo et al., 2002) or peripheral arterial disease (Baumgartner et al.,1998; Losordo et al., 2001; Rajagopalan et al., 2003). If athletes used these treatments for improving blood vessel production, the result could be a enhanced supply of oxygen and other nutrients to the tissues. With better supply, muscles, lungs, the heart and other parts of the body would not exhaust easily. As VEGF is already used in several clinical studies, VEGF gene doping would be possible at this time with the gene therapy vectors used in those studies. (Haisma H. J.,et al, 2004).

PGC1 (PPAR (peroxisome-proliferator-activated receptor) co-activator) and PPAR

In addition to oxygen delivery, metabolic characteristics of muscle fibres are important for strength. Studies in mice show that transgenic animals for either PGC1or PPARhave an increase in type I fibres as assessed by oxidative enzyme expression, muscle colour (which reflects myoglobin content), sarcomeric protein expression and mitochondrial content .Importantly, these mice show superior performance in running endurance and muscle fatigue resistance. The question remains whether hyper expression of these factors in adult animals, as opposed to during embryonic development, would have similar effects. ((Harridge S. And Vellosi C., 2008).

Adenosine monophosphate analogue (AMP)-(AICAR)

It is an analogue of adenosine monophosphate (AMP), the activator of AMP-dependent kinase (AMPK). Studies have shown that activated AMPK enzyme may reduce the level of anabolic processes, including synthesis of fatty acids and proteins, and increase the level of catabolic pathways such as glycolysis and fatty acid oxidation . So far, however, there have not been published any data on AICAR ergogenic effects in healthy and trained people. AICAR is also an experimental drug and is included in the WADA prohibition list (Brzeziańska E1 et al, 2014).

Phosphoenolpyruvatecarboxykinase (PCK1, PEPCK-C)

It is a key enzyme regulating gluconeogenesis. This enzyme is considered crucial in glucose homeostasis and is involved in the Krebs cycle]. Studies in mice have shown that its expression is associated with increased muscle endurance in animals. So far, there are no published literature data confirming the occurrence of side effects associated with transfer of the PCK1 gene or protein used as doping. Gene transfer as a method of strengthening the desired physical and physiological characteristics or improving the natural athlete phenotype is an attractive way to achieve success in sport for many athletes. For this reason, intensive investigations on the potential use of gene doping in many sports are nowadays increasing in number. (Brzeziańska E1 et al, 2014).

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