Gene Doping

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International Symposium

Rome, 15 October 2006
• 1964 Innsbruck Olympics: Finish Nordic Skier Eero Mantyranta 2 gold medals
  - mutation to produce more red blood cells.

• 2002 Salt Lake City Olympics: Spanish Nordic Skier Johann Muehlegg 1 gold and 2 silver medals
  - doping to produce more red blood cells.
Degrees of potential genetic intervention

• Genes and sports
  – Roger Bannister, first men to run one mile under 4 min.: “Athletes are not born equal”
    • West African: short distance
    • East African: marathon
    • Caucasians: swimming

• Gene doping: Gain advantage through gene transfer

• Genetic screening: Childs evolve top athletes

• Genetic manipulation: gain genetic predisposition
  • Marion Jones, Tim Montgomery: child, genetic advantage?
  • Steffi Graf, Andre Agassi: child, genetic advantage?
Gene therapy is a promising technique. Some first therapy trials: EPO, GH: link with sport is evident

**WADA position**

“the non-therapeutic use of cells, genes, genetic elements, or the modulation of gene expresión, having the capacity to enhance athletic performance, is prohibited in sport”

Probably not yet an actual problem. Gene therapy still mainly in clinical trials, but...

...what to do beyond Beijing 2008 Olympics?
• Gene Therapy
  – 3000 patients, some (few) side effects. Trials on going.
    • x-linked severe combined immunodeficiency disease
    • adenosine deaminase deficiency
    • haemophilia B
    • ..... 
  – Two registered products: Vitraene (antisense technology); tumor supress gene p53 (reduce tumor growth; China)
Sport authorities Concerns


• 2004 / 2005 WADA Conferences:
  • 1st Conference at Banbury
  • 2nd Conference in Stockholm
Stockholm declaration

• Gene therapy now represents a proven, although very immature and still experimental field of human medicine.

• Clinical research in human gene therapy is filled with many recognized and unrecognized pitfalls and dangers.

• The participation of physicians in gene transfer procedures that are not fully compliant with standards of human clinical research should be considered medical malpractice.

• Greater interactions should be encouraged to stimulate awareness of the potential illicit use of gene transfer techniques.

• New detection methods are likely to emerge and will help to prevent tainting of sport by gene doping. Research programs should be supported.

• The use of genetic information to select for or discriminate against athletes should be strongly discouraged.

• Sports organizations should promote knowledge about the potential dangers associated with the misuse of genetic manipulations.
Potential Sport Targets

- **EPO**: Gene therapy with EPO increases haematocrit in animals more than 80%
- **GH / IGF-1**: Promote muscle mass
- **VEGF** (Vascular endothelial growth factor): Increase blood supply
- **Myostatin** (negative regulator of muscle formation). Blockers increase skeletal muscle.
- **Endorphins** (for pain)
Laboratory mice at the University of Pennsylvania seem to hold the "beneficial" effects of IGF-1 throughout their lives.
Myostatin inhibition

In 1997, scientists McPherron and Lee revealed to the public the 'secret' of an anomaly that livestock breeders have capitalized since the late 1800's: the gene responsible for big beefy cows.

Transgenic rats

A German boy had mutation with lack of myostatin production

(Schuelke M, N Eng J Med 2004, 350: 2682-2688)
Ways to increase oxygen transport and delivery

- Blood transfusions
- Altitude training - hypoxic houses
- Modified haemoglobins
- Allosteric modifiers of haemoglobin
- Perfluorocarbons
- rHuEPO, mimetics and analogues
- Gene therapy with EPO genes
Cells expressing rmEPO
-C2C12 muscle-derived mouse cells were transfected with the pCMVmEpo expression vector, kindly provided by Dr. Evelyne Zeyra

- Subjected to G418 selection.

- Resistant cells were grown and assayed for EPO production (ELISA Quantikine R&D).

Scheme of the mEpo vector used in gene transfer studies
Awaiting for gene transfer

Muscle width measurement

Setting electrical parameters

Anaesthesia

Plasmid injection

Electroporation

Tibialis muscle exposed

Applying a conductive gel

Work done!
Effects after gene transfer to mice muscle

Serum EPO

Hematocrit
Gene therapy with EPO

Monkeys injected with a virus carrying the gene for EPO
Long-term pharmacologically regulated expression of erythropoietin in primates following AAV-mediated gene transfer

Victor M. Rivera, Guang-ping Gao, Rebecca L. Grant, Michael A. Schnell, Philip W. Zottick, Leonard W. Rozamus, Tim Clackson, and James M. Wilson

Repoxygen (Oxford Biomedica)

Viral gene delivery vector carrying the human EPO gene under the control of the hypoxia control element HRE. Prepared for clinical trials.

TECHNOLOGY
The hypoxia response element (HRE) is a proprietary gene switch which imposes oxygen mediated control on any linked gene.
The World Anti-Doping Code
The 2006 Prohibited List

PROHIBITED SUBSTANCES
• S1. Anabolic Agents
• S2. Hormones and related substances.
• S3. Beta-2 Agonists
• S4. Agents with anti-oestrogenic activity
• S5. Diuretics and other masking agents
• S6. Stimulants
• S7. Narcotics
• S8. Cannabinoids
• S9. Glucocorticosteroids

PROHIBITED METHODS
• M1. Enhancement of oxygen transfer
• M2. Chemical and physical manipulation
  • M3. Gene Doping

SUBSTANCES PROHIBITED IN PARTICULAR SPORTS
• P1. Alcohol
• P2. Beta-blockers
Possible strategies for detection gene doping

Screening
- Indirect physiological models
- Immune response to viral vectors
- DNA microarrays and proteomic profiling (biopsies or blood mononuclear cells)
- DNA bar codes (difficult, it depends on multiple parties)
- Different isoforms (promising for EPO)

Confirmation
- Confirmation by non invasive imaging detection of unexpected expression in an ectopic tissue
Non invasive molecular imaging of gene expression useful for doping control: Pilot study in animals after erythropoietin gene transfer

You may say I’m a dreamer, but I’m not the only one
Imagine, John Lennon, 1974

IMAGENE consortium
Biomedical Research Park, PRBB, Barcelona
IMIM, UPF, CRG, IAT
IMAGENE
Non invasive molecular imaging of gene expression useful for doping control: Pilot study in animals after erythropoietin gene transfer

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RESEARCH HYPOTHESIS AND OBJECTIVES

- Most gene transfer processes produce the expression of mRNA for the target hormone-protein in unusual cells or tissues.

- mRNA molecules will hybridize with suitable antisense modified oligonucleotides available to the tissue expressing the ectopic hormone-protein.

- If a label of appropriate energy is associated to the modified oligonucleotides, detection of the unusual hybridization may be carried out non-invasively in real-time by suitable imaging technologies.
IMAGENE Working Plan

- Selection of target EPO-mRNA fragments suitable for hybridization
- Synthesis of PNAs incorporating cell-penetrating peptides
- Labelling of PNAs with PET and SPECT emission atoms
- Transfer of EPO genes into mice muscle
- Verification of successful transfer and EPO gene expression in transfected mice
- Imaging of EPO gene expression *in vivo* by PET and/or SPECT after labeled-PNAs administration
TAT-PNA1 5 μM covalent binding
Inhibition of EPO expression in C2C12 transfected cells further incubated with concentrations of PNA1
INCORPORATION OF *I-PNA-TAT

Incorporation of $^{123}$I-PNA into cells

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</table>

Counts

- Control
- EPO
EXIT OF *I-PNA-TAT

Disposition of $^{123}$I-PNA (counts in cells)

Counts

Time (hours)

Control
EPO
Retention of radioactivity

Leg intensity (Right-Left/Left)

-0.2
-0.1
0
0.1
0.2
0.3
0.4
0.5
0.6

Control/PNA1
Transgenic/PNA1
Transgenic/PNA2
Final Aim: Sensitivity and Resolution of Positron Emission Tomography (PET) or Single Photon Emission Computerized Tomography (SPECT)
Conclusion

• Gene therapy may be soon among classical medical treatements
• There are several possibilities for sportmen to cheat by misusing this new therapeutic tool
• Efforts are under way to detect Gene Doping in the mid-term future