PROJECT REVIEW

"IMAGENE: Non-invasive molecular imaging of gene expression useful for doping control: extension study in animals after erythropoietin gene transfer"

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Non invasing imaging of gene expression will have a great potential usefulness in medicine (identify anomalous expression of genes related to diseases, verify the result of gene therapy applications, etc) as an alternative to invasive biopsy approaches. Positron emission tomography (PET) and single photon emission computerized tomography (SPECT) technologies appear as the most sensitive imaging tools. A very important field of application of imaging gene expression will be the prevention of the prohibited misuse of gene therapy in athletes. Some of the more studied approaches for imaging gene expression have been the imaging of reported genes, introduced purposely through a gene transfer process, or the detection of the expressed protein when it is an enzyme or a receptor. Neither of these characteristics take place in doped athletes, where the hormone (EPO, IGF-I, GH) is not an enzyme/receptor and where the practice is purposely attempted to be masked.

An alternative of wider applicability for imaging gene expression is the detection of the actual outcome of transfection, that is, the mRNA being formed in unusual tissues after the gene transfer process. This approach is independent of the construct and the vector used for gene transfer and is applicable to any gene transfected to tissues not usually expressing the protein, such is muscle for EPO.

The imaging of mRNA may be carried out by hybridization in the tissues of the mRNA molecules with suitable antisense oligonucleotide probes labeled for PET or SPECT detection. Some of the common problems when designing oligonucleotides for this purpose is the difficulty they may have in entering into the cell *in vivo* and the stability of the mRNA because of potential activation of RNAse H by the probe. In this regards, the recently developed Peptide Nucleic Acids (PNAs) appear as one of the best alternatives to other oligonucleotides such as phosporothioates, 2'-O-methyl RNAs or 2'-fluoro-arabino nucleic acids. PNAs are expected to have high stability, strong hybridizing properties, appropriate pharmacokinetic characteristics and the possibility to incorporate cell-penetrating peptides to allow cell entrance and accommodate positron or single photon emitting atoms (eg. ^{18}F, ^{11}C, ^{123}I) by relatively simple chemical treatments. In spite of all these advantages, the field of imaging gene expression by hybridization of mRNA by labeled oligonucleotides and analogs is not yet completely developed, and it is considered that a pilot project in animals (mice) is required before a more definitive effort be carried out for doping prevention.

Accordingly, the pilot project proposed, addressed to image the presence of transfected EPO genes into muscle of mice, will follow the following steps:

a) selection of target EPO-mRNA sequences suitable for hybridization
b) synthesis of antisense PNAs incorporating cell-penetrating peptides
c) in vitro verification of the stability and hybridizing properties of synthesized PNAs
d) labeling of PNAs with PET and SPECT emission atoms
e) transfer of EPO genes into mice muscle by electroporation
f) verification of successful EPO gene transfer and expression in transfected mice
g) imaging of gene expression in vivo by PET and/or SPECT after labeled-PNAs administration
h) conclusions and recommendations for further steps

Results and Conclusions

"IMAGENE Non invasive molecular imaging of gene expression useful for doping control: Pilot study in animals after erythropoietin gene transfer"

The project IMAGENE (Non invasive molecular imaging of gene expression useful for doping control: Pilot study in animals after erythropoietin gene transfer) was approved by WADA on October 2004 for an initial period of one year. The results of this first period were presented to the WADA Committe on Gene Doping in Stockolm on December 2005. As a consequence of the recommendations of the meeting, an extension of the project was approved on March 2006. In this final report, an update of the overall project is presented.

The hypothesis underlying the project was based on several assumptions. The first was that most of gene transfer processes produce the expression of an mRNA for the target hormone-protein in unusual cells or tissues. The second was that these mRNA molecules will hybridize with suitable antisense modified oligonucleotides such as PNAs introduced in the tissues expressing the ectopic hormone-protein. The third was that if a radiolabel of appropriate energy is associated to the modified oligonucleotides, the detection of the unusual hybridization may be carried out non-invasively from the outside of the body by suitable imaging technologies.

Thus, the global objective of the project was to develop a pilot study in animals (mice) to verify the hypothesis for further potential extrapolation of the approach to humans in the future.

The specific objectives were as follows
a) to develop modified oligonucleotides (peptide nucleic acids (PNAs) with cell penetration properties (Tat-PNAs) to hybridize mRNA expressing transfected EPO.
b) to label the Tat-PNAs with radiochemicals suitable for radiological external detection.
c) to evaluate in vivo the imaging capability (PET/SPECT) in animals expressing EPO in muscle after a gene transfer process
d) to develop recommendations for the development of similar procedures for the detection of EPO and other gene transferred doping hormone-proteins in humans.

All the objectives of the IMAGENE project (out of PET studies) were accomplished.