ANIMAL MODELS FOR GENE THERAPY

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Transfer of exogenous DNA to keratinocytes has been demonstrated to be a suitable method for gene therapy applications. This procedure has the following advantages with respect to the classical recombinant protein administration: no need of repeated injections, no need of protein purification and quality control, no danger of repeated administration of toxic contaminants and possibility of reaching an *in vivo* release of the active principle that mimics the physiological mechanism.

With basis on these assumptions, an *ex vivo* gene transfer protocol was utilized in our laboratory, in which primary human keratinocytes were transduced with an efficient retroviral vector (LXSN) encoding the human growth hormone gene. This procedure yielded the highest in vitro secretion level ever described in the literature for hGH. When the grafting of the epithelial sheet made with these genetically modified keratinocytes was implanted into the Little/Scid mouse, an animal model that joins dwarfism to severe immunodeficiency, a level of 1.5ng hGH/mL was detected in the serum, with a concomitant significant weight gain.

A parallel strategy has been carried out using the mouse growth hormone (mGH) gene for the purpose of studying a homologous system, which should provide a higher *in vivo* expression and a more evident phenotypic reversion. In this study, the modified keratinocytes presented a stable secretion level of up to 11 ng mGH/million cells.day, the highest ever reported for a form of GH in this type of cells.

Our most recent results on gene therapy have been published in 2003 in a very prestigious journal and more data, concerning a new grafting methodology, a long-term assay and the mechanism of GH release in vivo are ready to be published. Much higher circulatory levels (up to 20 ng mGH/mL) were found in the Little/Scid mice but, unfortunately, after a few hours, for some unknown reasons, mGH concentration fell down to baseline levels.

Our group, being considered a leader in this field, has recently been invited by the journal “Current Gene Therapy” to write an extensive review on the topic “Animal Model for Growth Hormone Gene Therapy”. The review, which analyzes 112 pages on the field, considers that the treatment of growth hormone deficiency via parenteral administration of recombinant hGH has greatly benefited from recombinant DNA technology allowing production of practically unlimited amounts of the pure hormone. However, an alternative approach that may lead to correction of the clinical defect is presented by hGH gene transfer into somatic cells of the patient, either *ex vivo* or *in vivo*. GH gene therapy has not reached the clinics yet, but several interesting and promising animal models for this treatment have been developed and studied. They are not only potentially useful for elucidation of the still unresolved of the still unresolved mechanism of sustained in vivo gene product delivery, but also for opening the way to therapy of other protein deficiencies which gene therapy may be the only viable option. This review article describes, analyzes and compares the major animal models of GH gene therapy that have been developed in the last two decades.

BIOLOGICAL EFFECT OF RADIATION ON CELLS: CYTOGENETIC, THERAPEUTIC AND MUTAGENIC ASPECTS

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Keywords: chromosome aberrations; ionizing radiations; human peripheral lymphocyte; germinative cells; micronucleus; comet assay.

Ionizing radiation is a very known genotoxic agent, but on the other hand it is a therapeutic modality used in medicine. Therefore, a better comprehension of the cellular response to ionizing radiation is of great value from the radiological as well as oncological viewpoints. The present project has been developed by the group in three interlinked aspects. In the first one (cytogenetic aspect) a comparative study of the effects of different radiation types and radionuclides in human and rodents cells has been carried out. Parameters as survival curve, proportion of affected cells, distribution of lesions, kinetic of cell proliferation and apoptosis are studied. So, cytogenetic (chromosome aberrations and micronuclei) and biochemical (comet assay) techniques were developed and standardized in our laboratory. The results obtained from either human lymphocytes or Chinese hamster ovary cells (CHO-K1) showed that Sr-90 beta radiation induced more DNA damage than Co-60 gamma radiation when the cells were analyzed immediately after exposures. Another aspect of the study (therapeutic approach) is to analyze the cytogenetic effects of radiopharmaceuticals used in nuclear medicine, for example Sm-153-EDTMP applied for pain relief of bone metastasis and I-131 administered to patients with thyroid diseases such as differentiated thyroid carcinoma with or without recombinant human thyrotropin hormone (Thyrogen or hTSH produced at IPEN) on blood lymphocytes. The data obtained in animal model (rats) showed that previous administration of rhTSH-IPEN or Thyrogen following I-131 treatment favour a higher amount of chromosome alterations after 24 hours and 7 days in relation with basal values when compared to those obtained by treatment with I-131 alone. The cytogenetic results obtained in rats are in concordance with an increase of thyroid radioiodine uptake when treated previously with the hormone. The aim was to determine the effectiveness of the rhTSH when proceeded by I-131 application. Apparently, there was no difference between the effects in rats peripheral blood lymphocytes of Thyrogen, an imported product, and those of hTSH produced at IPEN.

Another radiopharmaceutical that has been analyzed is radiolabeled [DOTA,Tyr3] octreotate. This drug is a somatostatin analogue peptide that bounds to tumors expressing sst receptors (such as neuroendocrine, central nervous system, breast, lung, pancreas, thyroid, colon, and lymphatic tissue tumors). Octreotate labeled with the beta particle-emitter is utilized with promising results for the identification and treatment of various kinds of tumors. Preliminary cytogenetic analysis showed that the effects of [DOTA, Tyr3] octreotate labeled with I-131 and Lu-177 were similar at cellular level.

These studies are searching for the optimization of therapeutic strategies and for offering a better quality of life to the patients. Finally, another aspect of the study (mutagenic approach) is on the effects of ionizing radiation in germinative cells of the intermediary host of Schistosomiasis, by the analysis of descendants of irradiated progenitors, considering mortality and embryonic malformation. Preliminary results showed that F1 generation had a high incidence of malformed embryos and that malformation types were not different from those observed when the embryos were irradiated directly with Co-60 (theratogenic effect of radiation).