Gene transfer to muscle by electroporation: control of transgene expression and effects on host tissue

Pernille Hojman, MSc

This PhD dissertation was accepted by the Faculty of Helath Sciences of the University of Copenhagen, and defended on May 16, 2007.

Official opponents: Hans Skovgaard Poulsen, Declan Soden, Ireland and Sten Lund.

Correspondence: Pernille Hojman,

E-mail: Pernille.Hoejman.Moeller@rh.regionh.dk.

Dan Med Bull 2007;54:232

ABSTRACT

The thesis treats the regulation of transgene expression after gene transfer by electroporation (electro gene transfer, EGT), and the effects of EGT on host tissue. EGT offers great promise for treatment of numerous diseases as EGT to muscle tissue is highly efficient, leading to long-term expression of the transferred genes. Systemic therapeutic levels of e.g. EPO have been achieved after transfer to a limited muscle mass. There are several therapeutic implications of this - including using muscle tissue as an endocrine protein factory.

We introduced genes under Tet-On responsive promoters into tibialis cranialis (TC) muscles using a combination of high voltage (HV) and low voltage (LV) pulses, a combination specially optimised for safe and efficient gene transfer. The Tet-On system ensured regulation of the transgene expression through doxycycline delivery. We developed a treatment approach whereby the transgene expression could be controlled through 1) the amount of DNA transferred, 2) the muscle mass transfected, 3) the doxycycline concentration, and 4) re-transfection. Results showed that: a) low DNA amounts were needed. In fact 0.5 g DNA to one TC muscle led to significant increases in haemoglobin levels – this amount extrapolates to 1.4 mg of DNA in humans, b) prescription of preset target haemoglobin levels was obtainable through different strategies, thus undershooting could be corrected by retransferring, while overshooting could be alleviated by reducing dose of doxycycline.

Furthermore we investigated the effects of EGT on muscles by microarray analysis, histology, expression of stress markers e.g. intracellular Ca^{2+} contents and ATP loss, transmembrane ion exchange and evaluation of functional impairments. Using the HV+LV pulse combination, we found small changes in the expression of cytoskeleton and intracellular transport proteins, but no other unintended changes in the gene expression profiles. At the cellular and physiological levels relatively small changes in Na^{+}, K^{+}, Ca^{2+} and ATP contents after EGT were found. These transient changes might explain the rapid recovery observed in the functional tests. Contrarily histological analysis revealed reversible morphological changes in some cells after 4 and 48 hours. These changes were more pronounced if DNA was present.

In conclusion the study showed that EGT with the HV+LV pulse combination is highly safe and efficient, offering the possibility of prescribing accurate preset therapeutic levels of the transgenic product; a prerequisite for introducing EGT into clinical use.