Ideal conditions for gene transfer through electroporation in dystrophic models for muscular dystrophies

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Keywords: Muscular dystrophy, Murine dystrophic model, Electroporation, Plasmid necked DNA and Transfer gene.

Electrical pulses for gene transfer have been successfully used to potential gene expression *in vivo*. The muscle tissue is the most widely used in electroporation, because of its accessibility and lasting expression of transgene. However, skeletal muscle also provides a natural barrier for transgene´s action, such as dense extracellular matrix and presence of fibrous regions as endomysium and perimysium areas. The dystrophic muscle is an especially adverse recipient for gene therapy, due to its intense process of degeneration followed by an increase in endomysial fibrosis and adipocyte infiltration, which are an inhibitor of both muscle function, and myogenic activities of satellite cells. The Dmd^{mdx} mouse is the model for Duchenne muscular dystrophy (DMD) with mutation in the dystrophin gene, with high regeneration on low connective tissue infiltration. The myodystrophy mouse ($Large^{myd}$) harbors a mutation in the glycosyltransferase Large, which leads to altered glycosylation of α-DG. It is a model for congenital CMD-1D, with high degeneration and connective tissue infiltration. These two mice models differ also in the severity of the phenotype. The aim of the present work is to achieve a highest level of efficacy for gene transfer throughout electroporation in animal models for muscular dystrophies. We studied two dystrophic models with variability in muscle degeneration intensity: the Dmd^{mdx}, $Large^{myd}$ and using C57Black as normal control. The muscle was surgically accessed, injected or not with hyaluronidase, injected with a plasmid expressing GFP, and followed by variable conditions of electroporation. We observed differences between the dystrophic versus the normal strain, as evaluated by the expression of GFP plasmid in the muscles. The Dmd^{mdx} model started showing muscle alterations caused by the electrical pulse since the voltage of 30V, increasing in a number with pulses of 50V, remaining similar in 100V. Positive GFP fibers were identified with 50V, increasing in the number with pulses of 100V. Height pulse volts are, therefore, beneficial in this model. On the other hand, in the more dystrophic $Large^{myd}$ muscles, electric pulse of 20V generated a larger degeneration with very weaker GFP expression, which remains weak also using higher voltages. Therefore, the more dystrophic muscles are also more resistant for this methodology. These results point to a potential use of plasmid necked DNA electroporation as a valuable tool for gene therapies in muscle diseases, since the suitable pulse power is adjusted for each murine dystrophic model.
Financial support: FAPESP-CEPID, CNPq-INCT, FINEP, ABDIM.