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Non-viral gene transfer to skin, muscle and liver for expression of growth hormone

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INTRODUCTION

DNA injections followed by electroporation led to sustained levels of circulating human growth hormone (hGH) after electrotransfer of hGH plasmids into the muscle of *immunodeficient dwarf* mice (*lit/scid*) and to a significant weight increase. In order to develop a clinical relevant gene therapy protocol we have started to compare a panel of growth hormone plasmids in normal mice (NRMI), with different promoters, transferred to skin or muscle using different electro-transfer equipment .

AIM

The present study aimed to compare the differences between the promoters, UBI-C vs. CMV, the hGH genomic vs. cDNA, intradermal vs. intramuscular injections, and also the equipments Cliniporator/IGEA vs ECM830/BTX.

MATERIAL AND METHODS

NRMI mice were injected intramuscular and intradermally with the plasmids puC-UBI-ghGH, containing the UBI promoter and genomic hGH, and the pCMV-ghGH and pCMV-chGH, containing the CMV promoter and the genomic and cDNA, followed by electroporation. The electric pulses were administered with custom made plate electrodes connected to the electroporator Cliniporator/IGEA and/or ECM830/BTX, and the conditions for muscle electroporation were: 1 high voltage (HV) pulse of 800V/cm with length of 100µs and 1 low voltage (LV) pulse of 100V/cm with 1 s of lag between the HV and LV. For skin electroporation: 1 high voltage (HV) pulse of 1000V/cm with length of 100µs and 1 low voltage (LV) pulse of 100V/cm with 1 s of lag between the HV and LV. The electrodes were coated with electrogel and the electric pulses were delivered 1 minute after the plasmid injection. An hydrodynamic injection of puC-UBI-ghGH was utilized as a positive control of hGH secretion. As controls EPO plasmids (puC-UBI-EPO and pCMV-EPO) were injected intramuscular and compared the different promoters UBI-C vs. CMV.

RESULTS

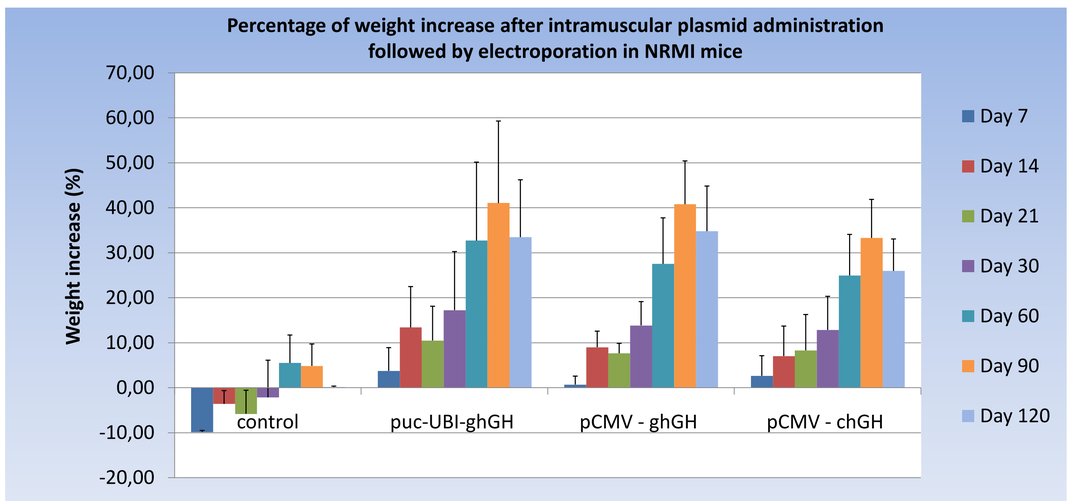


Fig. 1 : Gene electrotransfer with different promoters in NMRI mice. 10 µg of plasmid were injected intramuscular and all the groups that received the GH gene had an weight increase percentage approximately of 40% when compared to the control group. The last 2 groups show a comparison between genomic and complementary hGH gene. (n=4 animlas/group)

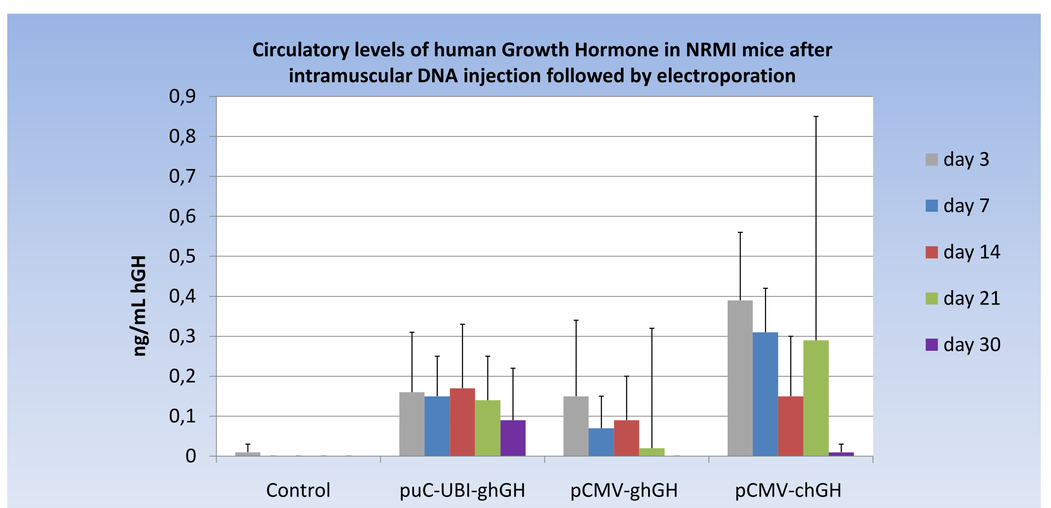


Fig. 2: Circulatory levels of hGH in NRMI mice after intramuscular injection of different plasmids containing the UBI and CMV promoter, and also a comparison of genomic vs. complementary DNA. (n=4 animals/group)

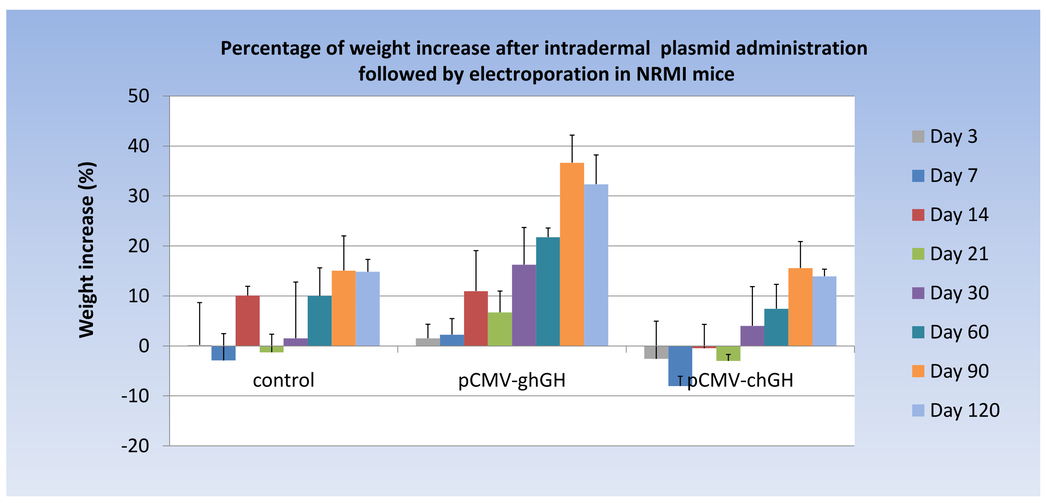


Fig. 3: Gene electrotransfer to skin, comparing the CMV genomic vs. complementary hGH gene. For intradermal delivery, we administered 2 injections of 75 µg of plasmid in the dorsal region. Control group was injected with saline. Circulatory levels were not found in that serum samples until 30 days after injections. (n=4 animals/group)

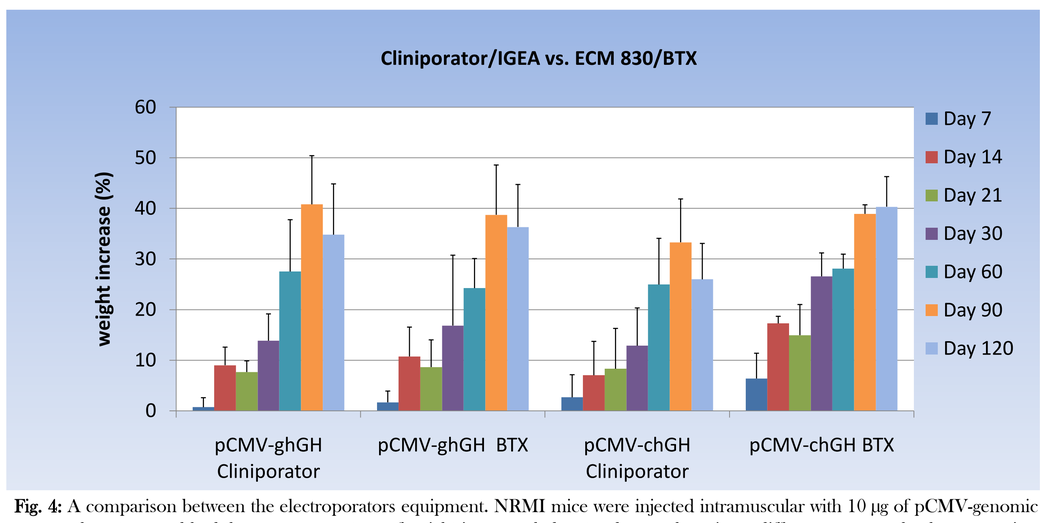
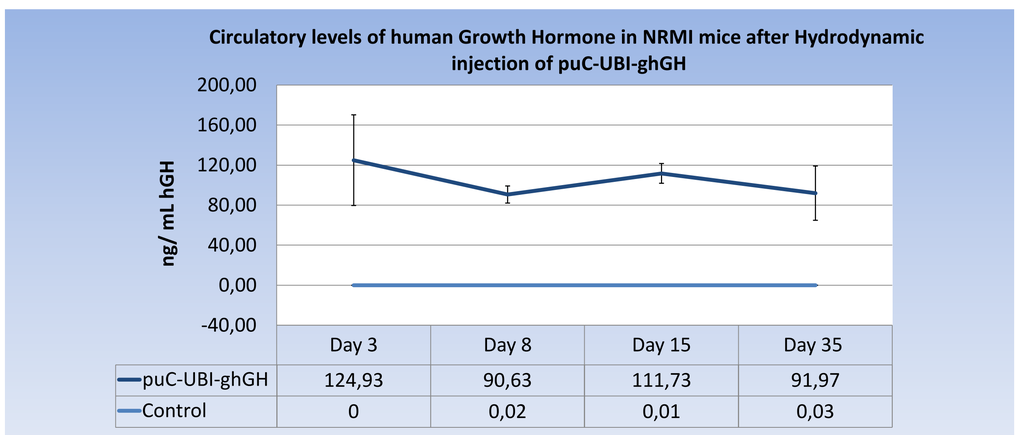
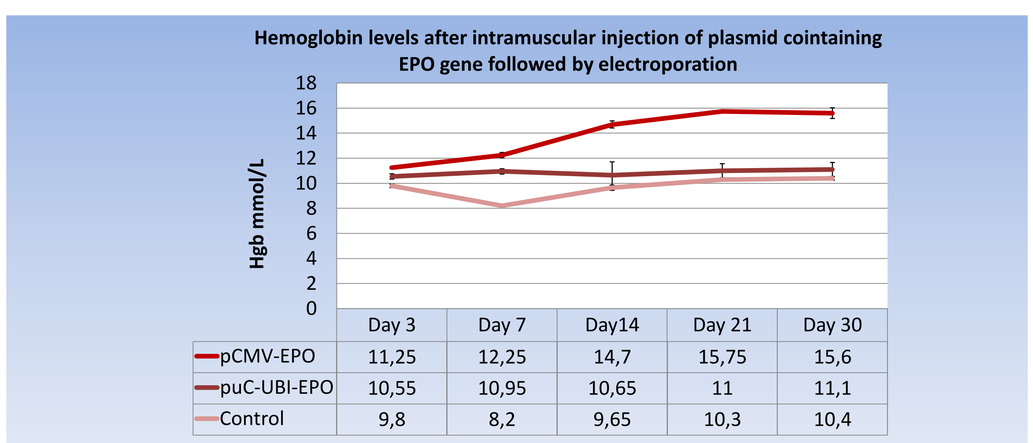


Fig. 4: A comparison between the electroporators equipment. NRMI mice were injected intramuscular with 10 µg of pCMV-genomic or complemetary, and had the same percentage of weight increase, led us to observe there is no difference to muscle electroporation utilizing the hGH gene. (n=4 animals/group)



	Day 3	Day 8	Day 15	Day 35
puC-UBI-ghGH	124,93	90,63	111,73	91,97
Control	0	0,02	0,01	0,03

Fig. 5: Hydrodynamic injection of 75µg of puC-UBI-ghGH plasmid diluted in ringer solution. The control group received administration of ringer solution. The gene transfer to the liver can produce a large amount of protein and also have a long term of expression. (n=2 animals/group)



	Day 3	Day 7	Day14	Day 21	Day 30
pCMV-EPO	11,25	12,25	14,7	15,75	15,6
puC-UBI-EPO	10,55	10,95	10,65	11	11,1
Control	9,8	8,2	9,65	10,3	10,4

Fig. 6 : Comparison between CMV and UBI plasmids containing the EPO gene administered in the muscle of NRMI mice. Injection of 2,5µg of plasmid followed electroporation, and saline injection for the control group. (n=2 animals/group)

CONCLUSIONS

Gene electrotransfer of a plasmid containing hGH gene lead to an increase of weight of *normal* mice (fig. 1 & 2). Intradermal injection of human genomic and cDNA gene showed same level of weight increase, although circulatory levels of human growth hormone could not be detected until day 30 (fig. 3). The two different kind of electroporation equipment performed similar (fig.4). The plasmid with UBI promoter and human genomic growth hormone gives a solid expression after hydrodynamic injection (fig. 5), but the same promoter UBI does not work in the muscle with the EPO gene when compared to CMV (fig. 6).

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