SUSTAINED hGH EXPRESSION AFTER ELECTROTRANSFER OF NAKED DNA INTO DWARF "LITTLE" MOUSE SKELETAL MUSCLE

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Ex vivo growth hormone gene therapy based on retrovirally transduced primary human keratinocytes has led, in our previous work, to relatively high circulating levels of human (hGH) or mouse growth hormone (mGH) in "little" mice (Peroni et al., *J Gene Med* 2008; 10: 734-743). Unfortunately these levels fell to baseline within a short period of time. The use of non viral vectors is an alternative to the *ex vivo* Gene Therapy based on viral vectors. Non viral vectors are safer and less expensive, but also less effective than viral vectors. Electroporation, or electrotransfer, is a strategy used to increase the delivery of plasmid DNA intramuscularly or intradermically. This study aimed to verify the feasibility of the electrotransfer technique for increasing the efficiency of the intramuscular injection of DNA, using a plasmid containing the hGH gene. The pUC-UBI-hGH plasmid used in this study contains the ubiquitin C promoter and the genomic hGH sequence. "Little" mice (lit/lit) were anesthetized followed by a hyaluronidase injection (20 U / 50 µL) in the quadriceps muscle region. After 30 minutes, different amounts (12, 5; 25; 50; 75; 100µg) of purified plasmid DNA were administered, followed by electrotransfer using eight 50V pulses of 20 ms and 0.5 s of interval (Fig.1). Blood was collected from the retro-orbital cavity after three days and hGH levels in the sera were determined by radioimmunoassay. We can observe that the dose-response curve presented a highly significant correlation ($P<0.01$) in the 0-50 µg range. In a second experiment, 50 µg of purified plasmid were administered as described, blood being collected after 1, 3, 6, 9 and 12 days. According to preliminary data, circulating levels of 2-3 ng hGH/mL were maintained for at least 12 days in the immunocompetent "little" mouse (Fig. 2). Such hGH levels have never been reported for naked DNA muscle injection. We intend now to verify the period of permanence and *in vivo* bioactivity. In conclusion, we can say that the use of this methodology is quite promising for the development of new models of *in vivo* gene therapy for growth hormone.

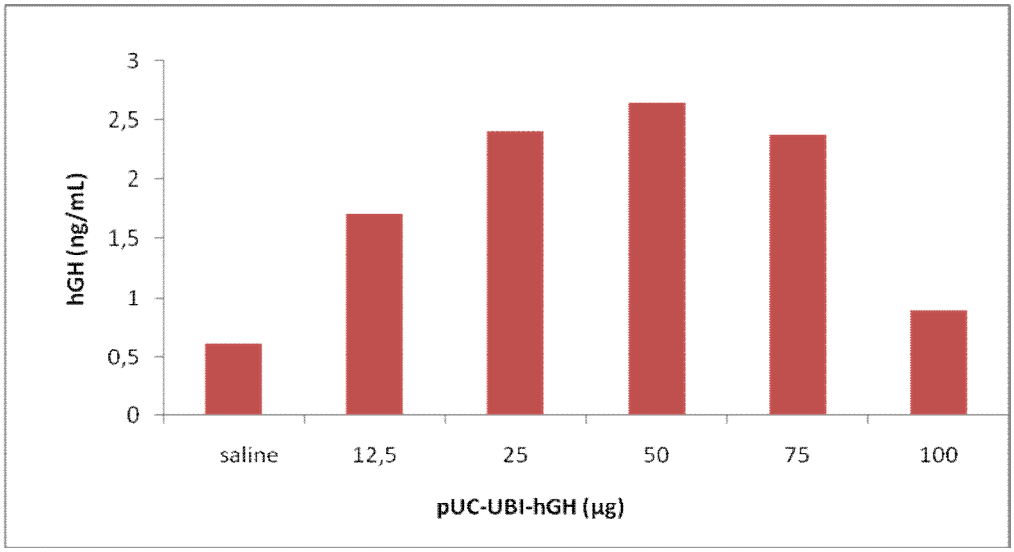

Figure. 1: hGH levels in sera from *lit/lit* mice after three days (n = 3 animals per dose) from the injection of different amounts of plasmid DNA, followed by electrotransfer using eight 50V pulses of 20 ms with 0.5s of interval. The dose-response curve, from 0 to 50 µg DNA, was: Y=0.036X+1.11 r=0.806 P<0.01 (n=11).

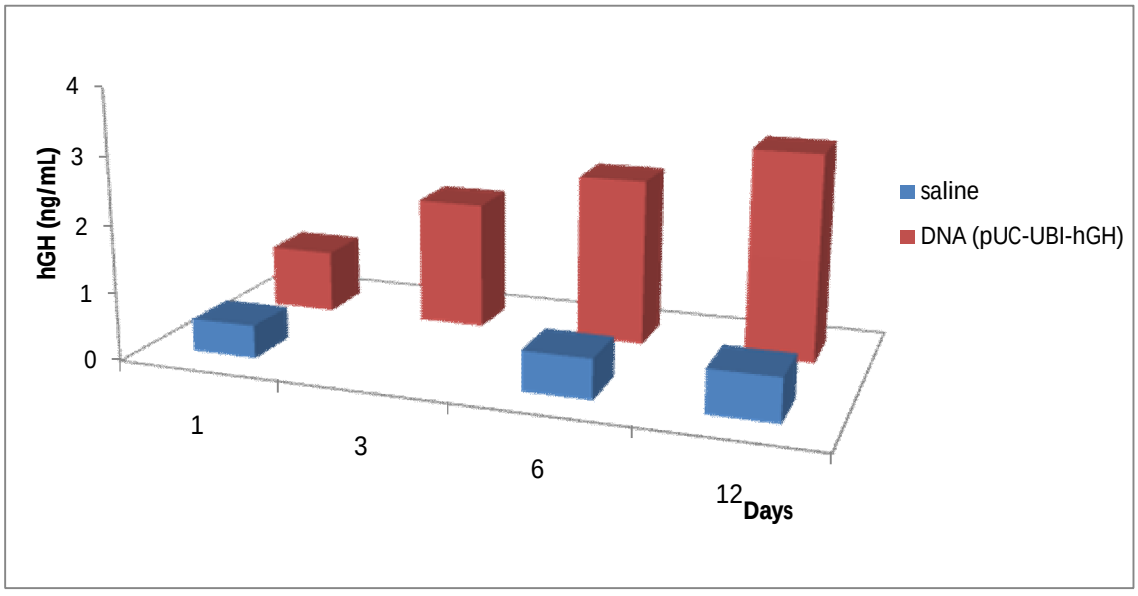

Figure. 2: Expression of hGH after administration of 50µg of pUC-UBI-hGH plasmid followed by electrotransfer, using eight 50V pulses of 20 ms with 0.5 s of interval, in little mice (n=3 animals/condition). Each animal was used once and then sacrificed.

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