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GROWTH PARAMETERS AFTER INTRAMUSCULAR hGH PLASMID ADMINISTRATION COMPARED TO RECOMBINANT hGH INJECTIONS IN LIT/SCID MICE

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BACKGROUND

We have previously obtained sustained levels of circulating human growth hormone (hGH) after electroporation of naked DNA in the muscle of immunodeficient dwarf (lit/scid) mice and these treated animals presented a highly significant weight increase of ~33% (Oliveira NAJ et al., *The Journal of Gene Medicine* 2010; 12: 580-585).

Electroporation, or electrotransfer, is a strategy used to increase the delivery efficiency of non-viral vectors (plasmid DNA) in different tissues or organs. The cell membrane, made up of phospholipids, is not normally permeable to charged molecules as DNA. *In vivo* electroporation involves the application of short, controlled, electric pulses to target tissue into which plasmid DNA has been injected resulting in increased permeability due to formation of pores. In this way, DNA molecules that cannot normally penetrate the cell membrane are allowed to enter the cell after administration of an electric pulse.

OBJECTIVES

This study aimed at confirming the efficacy of our model for *in vivo* gene therapy based on electrotransfer of hGH-coding plasmid DNA using different growth parameters and comparing this strategy to the conventional daily recombinant hGH injections in lit/scid mice.

METHODOLOGY

Animals

The mutant strain of B6 mice, CB17-Ghrhr lit/+ Prkdc scid/Bm (*lit/scid*) was obtained from Dr. W. Beamer (The Jackson Laboratory, Bar Harbor, ME, USA).

Plasmid

The pUC-UBI-hGH plasmid was kindly provided by Dr. T. G. Jensen (The Kennedy Institute, Glostrup, Denmark). This plasmid contains the ubiquitin C promoter and the genomic human growth hormone (hGH) sequence. The plasmid was transformed into DH5α *E. coli* competent cells using standard procedures. Plasmid purification was carried out using the Quiagen maxi-prep purification system (Hilden, Germany).

Plasmid administration and electroporation

The animals were anesthetized with xylazine and ketamine followed by a hyaluronidase injection (20 U/50 µL) into the right quadriceps muscle region. After 30 minutes, 50 µg of purified plasmid were administered in the same region, followed by electrotransfer, using an in-house apparatus with 8 pulses of 20 ms and 0.5 s of interval with 50 V. Saline was used as control in all assays.

Bioassay procedures

Two groups of lit/scid mice were submitted to electroporation. One (n=10 animals) received a single dose of 50 µg of pUC-UBI-hGH and the other (n=8 animals) saline. A third group (n=11 animals) received intraperitoneal injections of recombinant hGH (5 µg r-hGH/twice a day/animal). The body weight of the animals was determined throughout the entire assay period and used to calculate the average daily weight variation. Tail and nose-to-tail lenghts were determined at the end of the experiment, when internal organs (quadriceps and gastrocnemius muscles, kidneys, spleen, liver and heart) were also dissected and weighed. Serum mIGF-I was measured using the *Quantikine mouse-rat IGF-I* kit (R&D Systems, MN, USA). Significance was evaluated according to the Student´s *t* test .

RESULTS

Growth curves of lit/scid mice with a single DNA injection or saline, followed by electroporation, or with daily injections of recombinant hGH (r-hGH) are represented in Fig. 1. The linear correlation was highly significant (P<0.0001) for all treatments, and the slopes of the growth curves for animals treated with hGH-DNA or hGH-protein were very similar (0.094 g/mouse/day x 0.095 g /mouse/day). As we can observe in Table 1, the percentage of body weight increase was 22.3% for the DNA-treated group and 35.5% for the r-hGH injected animals. There were also significant increases in the tail and nose-to-tail lengths.

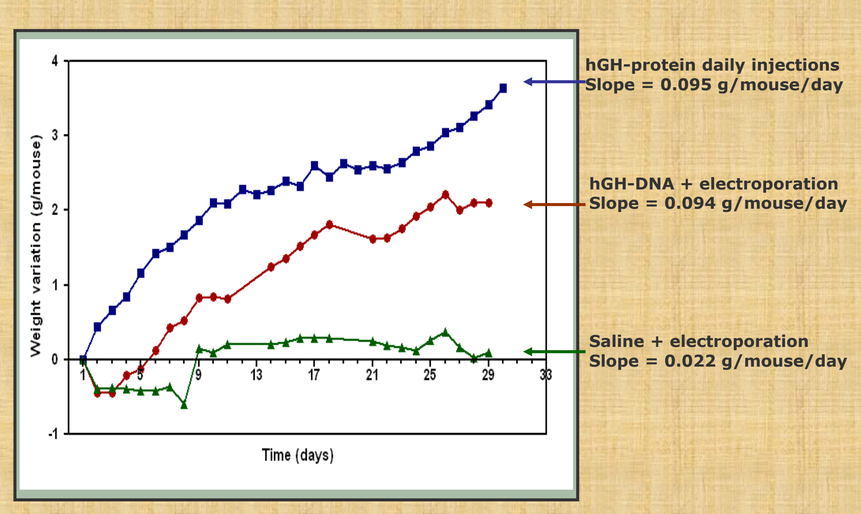

Fig. 1: Weight variation of lit/scid mice treated only once with 50 µg of pUC-UBI-hGH (—●—) or saline (—▲—), followed by eletroporation, or injected with recombinant hGH (—■—) (5 µg/twice a day/animal). Equation for each treatment:
- pUC-UBI-hGH (DNA): y= 0.094 x – 0.317 (n= 25; r= 0.965; P < 0.0001);
- recombinant hGH (protein): y= 0.095 x + 0.707 (n= 30; r= 0.949; P < 0.0001);
- saline: y= 0.022x – 0.318 (n= 25; r= 0.697; P < 0.0001).

Table 1: Growth parameters of lit/scid mice 29 days after a single intramuscular administration of 50 µg of pUC-UBI-hGH or saline, followed by electroporation, or after daily injections of r-hGH (5 µg/twice a day/animal)

Growth parameter	Before treatment (mean ± SD)	After treatment (mean ± SD)	Increase (%)	Statistical significance *1
Body weight (g)				
Saline	8.74 ± 1.14	8.61 ± 1.34	-1.5	n.s.*2
pUC-UBI-hGH	9.41 ± 1.24	11.51 ± 1.71	22.3	P<0.01
r-hGH	10.23 ± 0.92	13.86 ± 0.95	35.5	P<0.001
Tail lenght (cm)				
Saline	5.60 ± 0.32	6.10 ± 0.44	8.9	P<0.05
pUC-UBI-hGH	5.72 ± 0.26	6.27 ± 0.21	9.6	P<0.001
r-hGH	n.d.*3	6.57 ± 0.38	___	___
Nose-to-tail lenght (cm)				
Saline	11.85 ± 0.61	12.63 ± 0.68	6.6	n.s.*2
pUC-UBI-hGH	12.08 ± 0.61	13.24 ± 0.69	9.6	P<0.005
r-hGH	n.d.*3	13.75 ± 0.53	___	___

*1Significance level between after and before treatment; *2non-significant (P>0.05); *3non-determined.

Most of the organ weight of lit/scid mice treated with hGH-coding naked DNA or r-hGH showed significant increase compared to the control group (Fig. 2).

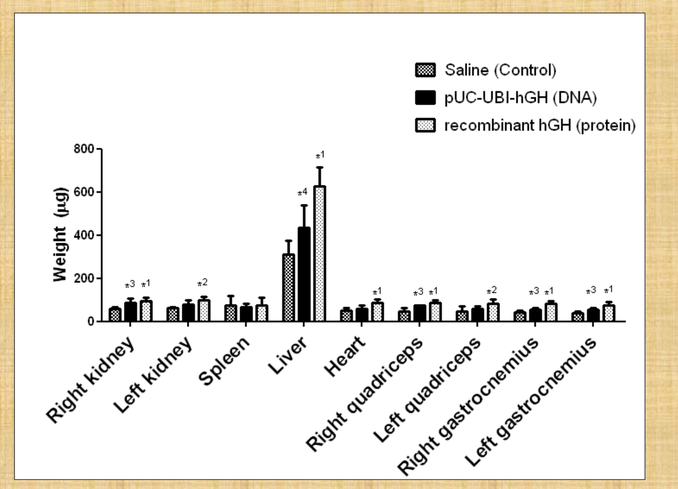

Fig. 2: Weights of dissected organs obtained from lit/scid mice 29 days after a single administration of pUC-UBI-hGH or saline, followed by electroporation, or after daily injections of r-hGH (5 µg/twice a day/animal). Significance levels between DNA or protein treatment and saline: *1 P<0.001; *2P<0.005; *3P<0.02; *4P<0.05; when not indicated P>0.05 (non-significant).

Plasma mIGF-I was determined on samples collected on day 7 and 29 (Table 2). As we can observe, there were significant rises in the circulating levels of mIGF-I between treated groups (DNA or protein) and control animals, with a tendency of a higher increase of this growth hormone mediator for the naked DNA-treated animals.

Table 2: mIGF-I concentrations determined in plasma of lit/scid mice 7 and 29 days after a single intramuscular administration of the hGH-expressing plasmid or saline, followed by electroporation, or after twice daily 5 µg injections of r-hGH

Animal group	mIGF-I (ng/mL)	C.V. (%)
Lit/scid – 7 days		
Saline	14.0 ± 3.3	23.6
pUC-UBI-hGH	94.2 ± 15.1	16.0
r-hGH	39.3 ± 8.1	20.6
Lit/scid – 30 days		
Saline	17.3 ± 1.8	10.5
pUC-UBI-hGH	92.1 ± 20.7	22.5
r-hGH	73.4 ± 27.1	36.0
Scid without treatment	371.4 ± 45.6	23.0

CONCLUSION

Intramuscular naked hGH DNA administration showed to be effective for promoting the growth of dwarf little mice and avoids the drawbacks of daily injections of the recombinant protein in the conventional treatment for patients suffering of GH deficiency (GHD). Quite interesting are the higher mIGF-I values shown by DNA administration, which deserve further studies.

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