GROWTH RESPONSES FOLLOWING A SINGLE INTRA-MUSCULAR hGH PLASMID ADMINISTRATION COMPARED TO DAILY INJECTIONS OF hGH IN DWARF MICE

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BACKGROUND

Plasmid-based gene therapy has proven to be particularly effective when non-viral gene transfer to muscle tissues is followed by electroporation, with potential applications to the treatment of different systemic diseases such as hemophilia, chronic anemia, diabetes mellitus and peptide hormone deficiency. Several studies, based on naked DNA administration, are already in phase I/II clinical trials for erectile dysfunction, metastatic melanoma, squamous cell carcinoma and myocardial or critical limb ischemia. As far as we know, no clinical trial is being carried out yet for the treatment of systemic diseases based on naked DNA administration.

In previous work, sustained levels of circulating human growth hormone (hGH) and a highly significant weight increase were observed after electrotransfer of naked plasmid DNA (hGH-DNA) into the muscle of immunodeficient dwarf mice (lit/scid) (Oliveira NAJ et al., *The Journal of Gene Medicine* 2010; 12: 580-585).

OBJECTIVES

In this study the efficacy of our model for *in vivo* gene therapy based on electrotransfer of hGH-coding plasmid DNA was compared to daily injections of recombinant hGH (r-hGH) protein, as assessed on the basis of several growth parameters.

METHODOLOGY

Animals

The mutant strains of CB17-Ghrhr lit/+ Prkdc scid/Bm (lit/scid) and scid mice were obtained from Dr. W. Beamer (The Jackson Laboratory, Bar Harbor, ME, USA). Normal mice (balb-C) were also used as control animals.

Plasmid

The plasmid pUC-UBI-hGH contains the ubiquitin C promotor upstream to a 2152 bp fragment of the hGH gene with 4 introns and polyadenylation sequences. This plasmid was multiplied in DH5-α bacteria and purified using the Qiagen 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 exposed right quadriceps muscle region. After 30 minutes, 50 µg of purified plasmid were administered in the same region, followed by electrotransfer with eight 50-V pulses of 20 ms, separated by 0.5 s intervals, using an equipment of in-house construction. Saline was used as the control in all assays.

Bioassay procedures

Two groups of lit/scid mice were submitted to electrotransfer. 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, for 28 days). The body weight of the animals was determined throughout the entire assay period and used to calculate the average daily weight variation. The tail and nose-to-tail lenghts were determined before and at the end of the experiment, when blood was collected and selected internal organs (quadriceps and gastrocnemius muscles, kidneys, spleen, liver and heart) were dissected and weighed. Serum mIGF-I levels were measured using the *Quantikine mouse-rat IGF-I* kit (R&D Systems, MN, USA). The concentration of glucose was determined with the Glucose PAP Liquiform System (Labtest, MG, Brazil). A P value < 0.05 was considered to be statistically significant.

RESULTS

The 28-day growth curves of lit/scid mice treated with r-hGH, hGH-DNA and saline are presented in Figure 1. It is interesting to note the practically identical slopes of the first two curves (P>0.05) and the slightly positive slope of the control curve, due mostly to recovery from electroporation.

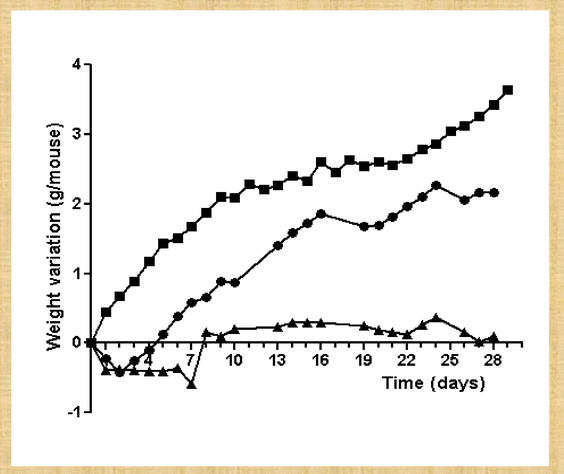

Figure 1: Weight variation of lit/scid mice treated only once with 50 µg of pUC-UBI-hGH (—•—) or saline (—▲—), followed by eletroporation, or injected with recombinant hGH (—■—) (5 µg/twice a day/animal). Equation for each treatment:
- pUC-UBI-hGH (DNA): y= 0.094 x – 0.317 (n= 25; r= 0.965; P < 0.0001);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

As we can observe in Table 1, the percentage of body weight increase was 23.1% 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.

Table 1: Growth parameters of lit/scid mice 28 days after a single intramuscular administration of 50 µg of hGH-DNA 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	8.53 ± 0.98	8.61 ± 1.34	0.9	n.s.
hGH-DNA	9.35 ± 1.25	11.51 ± 1.71	23.1	P<0.01
r-hGH	10.23 ± 0.92	13.86 ± 0.95	35.5	P<0.001
untreated scid*3		19.18 ± 2.42		
Tail length (cm)				
saline	5.60 ± 0.32	6.10 ± 0.44	8.9	P<0.05
hGH-DNA	5.72 ± 0.26	6.27 ± 0.21	9.6	P<0.001
r-hGH	5.66 ± 0.29*2	6.57 ± 0.38	16.1	P<0.001
untreated scid*3		7.96 ± 0.44		
Nose-to-tail length (cm)				
saline	11.85 ± 0.61	12.63 ± 0.68	3.8	n.s.
hGH-DNA	12.08 ± 0.61	13.24 ± 0.69	9.6	P<0.005
r-hGH	11.96 ± 0.61*2	13.75 ± 0.53	15.0	P<0.001
untreated scid*3		16.23 ± 0.61		

*1Significance level comparing groups before and after treatment; n.s., non-significant (P>0.05); *2Average value, calculated from saline and hGH-DNA pre-treatment groups; *3Same age as lit/scid after treatment.

The weight increases of several organs and tissues were 1.3-fold (right kidney) to 4.6-fold (heart) greater for protein than for DNA administration, which gave a generally more proportional growth (Figure 2).

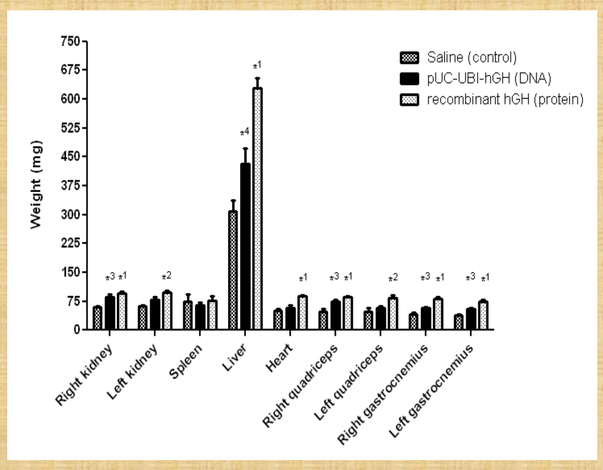

Figure 2: Weights of dissected organs obtained from lit/scid mice 28 days after a single administration of pUC-UBI-hGH or saline, followed by electroporation, or after daily injections of r-hGH (5 µg/twice a day/animal). Significance levels between DNA or protein treatment and saline: *1 P<0.001; *2P<0.005; *3P<0.02; *4P<0.05; when not indicated P>0.05 (non-significant).

Plasma mIGF-I showed a greater increase over the control with DNA (5- to 7-fold) than with protein (3- to 4-fold) administration (Table 2). Glucose levels were apparently unaffected, suggesting the absence of effects on glucose tolerance (data not shown).

Table 2: Plasma mIGF-I concentrations for lit/scid mice after a single intramuscular administration of the hGH-expressing plasmid or saline, followed by electroporation, or after daily injections of r-hGH during 28 days

Animal group	mIGF-I (ng/mL) ± SD	C.V. (%)	Statistical significance*1	Statistical significance*2
Lit/scid (7 days)				
saline	14.0 ± 3.3	23.6	___	___
hGH-DNA	94.2 ± 15.1	16.0	P<0.001	P<0.005
r-hGH	39.3 ± 8.1	20.6	P<0.01	
Lit/scid (28 days)				
saline	17.3 ± 1.8	10.5	___	___
hGH-DNA	92.1 ± 20.7	22.5	P<0.001	n.s.
r-hGH	73.4 ± 27.1	36.9	P<0.005	
Lit/scid (50 days)				
saline	27.2 ± 8.1	29.8	___	
hGH-DNA	119.3 ± 19.0	15.9	P<0.001	
without treatment	24.0 ± 0.61*2	27.9	n.s. *3	
untreated scid*4	371.4 ± 45.6	12.3	P<0.001*5	
untreated balb-C*4	398.6 ± 107.6	27.0	P<0.001*5	

*1Significance level comparing the hGH-DNA or r-hGH groups with the control; n.s., non-significant (P>0.05); *2 Significance level comparing the hGH-DNA and r-hGH groups; C.V., coefficient of variation; *3 Significance level comparing lit/scid without treatment with those treated with saline; *4 Same age as lit/scid at the end of the experiment; *5 Significance level comparing scid or balb-C mice without treatment and lit/scid treated with hGH-DNA after 50 days.

CONCLUSION

A gene transfer strategy based on a single hGH-DNA administration thus appears to be comparable to repeated hormone injections for promoting growth and may represent a feasible alternative for the treatment of growth hormone deficiency.

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