An *In vivo* Assay for Biological Activity of Synthesized Recombinant Human Growth Hormone

Short communication

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Summary

The biological activity of the recombinant growth hormone (GH) synthesized in The National Research Center for Genetic Engineering and Biotechnology (NRCGEB) was evaluated *in vivo*. In this regard a hypophysectomized rat model was required. Using a para pharyngeal approach, the sphenoid bone of rats was drilled and the hypophysis was out successfully by aspiration. Following a 7-day recovery period, rats were injected subcutaneously either with the recombinant human GH or the Novo, as standard. The results show that both hormones have an equal biological activity and resulted in a 100% growth increment equal to that in untreated intact rats indicating that the recombinant GH can be introduced as a good replacement of endogenous GH.

Key words: recombinant human growth hormone, hypophysectomy, rat

Introduction

Growth hormone (GH) is an adenohypophyseal hormone, which results in growth increment. GH, secreted from the pituitary gland is regulated by growth hormone releasing hormone of the hypothalamus (GhRH) with two known genes hGH-N and hGH-V. It is essential for body growth during postnatal life, influencing on longitudinal bone growth and cell mass of internal organs (Groesbeck *et al* 1987, Issakson *et al* 1985). GH receptors are found in many organs especially in liver and muscle where the insulin-like growth factor (IGF) and somatomedins are

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synthesized (Underwood *et al* 1988). GH stimulates the differentiation of prechondrocytes into cells that produce IGFs and respond to the mitogenic activity of these substances (Daughaday *et al* 1981, Eden *et al* 1983). GH also stimulates protein synthesis, increases the insulin level and lipid mobilization (Underwood *et al* 1988).

In vivo and *in vitro* assays were performed to investigate the biological activity of the human GH (hGH) (USP forum 1992, Roswall *et al* 1996 Zhu *et al* 1997). Despite *in vivo* assay, *in vitro* is time-consuming and expensive assay. To investigate the endocrine activity of hGH genetically or surgically manipulated animal models are required. Genetically deficient models are dwarf animals unable to synthesize their natural GH, which can be corrected by exogenous hGH. Surgically deficient models are those with the pituitary excised (hypophysectomized). Hypophysectomized rats are the best models for *in vivo* analysis of GH biological activity (Roswall *et al* 1996, Zhu *et al* 1997).

Using a surgical procedure to generate hypophysectomized rats we sought to determine whether the recombinant hGH (NRCGEB) promotes growth in growth-ceased hypophysectomized rats. Importantly, small but critical improvements, such as intubation during the operation and post-operational oxytetracycline therapy were made to facilitate the process.

Materials and Methods

Recombinant GH. The NRCGEB hGH was synthesized by *E.coli* strain DH5α carrying plasmid contained cDNA of hGH under the control of promoter and an ampicilin resistant gene. Purity of this recombinant GH was analyzed and confirmed by HPLC and gel electrophoresis, and compared with Novo, a commercially available hGH. The outcome of this comparison shows that they have similar biochemical properties (data not shown).

Animals. Twenty 4-6-week-old female Sprague Dawley rats (110-115 g) were

obtained from Razi Institute (Karaj, Iran) and divided in three main groups: intact control, sham-operated and hypophysectomized rats each of four.

Parapharyngeal hypophysectomy. The procedure was followed by the Waynforth and Flecknell, (1992) method. Each rat was anaesthetized with 0.15ml of a solution which contained ketamine:rampun: 0.9% sodium chloride (2:1:1). The procedure was performed using sterilized surgical instruments. Rats were then laid on back, taped and intubated using a cutdown catheter (No.5), essential for breathing during the operation. Following a midline incision, the trachea was exposed. All subsequent steps in the operation was then performed under a magnifier loop with a fluorescent light on top. The sterno-hyoid and the digastric muscles were retracted with forceps and the area was widened until the white surface of the bone was seen. The forceps are now scraped forcefully along the bone in an anterior direction for about 3-4mm to remove adhering muscle tissue, until a slope is felt. At top of this slope, a suture line often blue in color is seen. Drilling is started with a bone burr held vertically and precisely in midline and infront of the blue suture line. Drilling should proceed until the needle easily breaks through the bone. The hole is widened until most of the underlying gland is exposed. The curved tip of a suction pipette is applied to the drill hole and the pinkish pituitary gland is sucked. Skin was sutured and sanitized with an iodine solution.

Animal post-operational care. For a complete recovery, the operated animals including the sham-operated and hypophysectomized rats received 0.1mg/kg of oxytetracycline intraperitoneally for several days. Hypophysectomized rats received ACTH (40mg/100g B.W) and TSH (4.5 mg/100g B.W) intraperitoneally for 3-4 days.

Experimental design. The hypophysectomized group were divided into three subgroups each of four according to their treatment. The first treated subgroup was received Novo GH (0.8IU=0.3mg/rat), the second NRCGEB GH (0.3mg/rat) and

third phosphate buffered saline (PBS). Control and sham-operated rats received PBS. Except the intact control, the other groups were subcutaneously injected, daily for 7 days. Due to less technical complications, para-pharyngeal approach was chosen as the surgical method. Special post-operational care such as TSH and ACTH replacement therapy as well as antibiotic treatment was considered to overcome the high mortality and sickness. Rats were weighed every day and the growth curves drawn following a 7-day period. Data were analyzed using student-*t* test and ANOVA.

Results and Discussion

In spite of weight gain in sham-operated rats (22.26±6.48g) there is no significantly difference ($P<0.05$) between the group and intact control group (29.4 ±3.95g) (Figure 1).

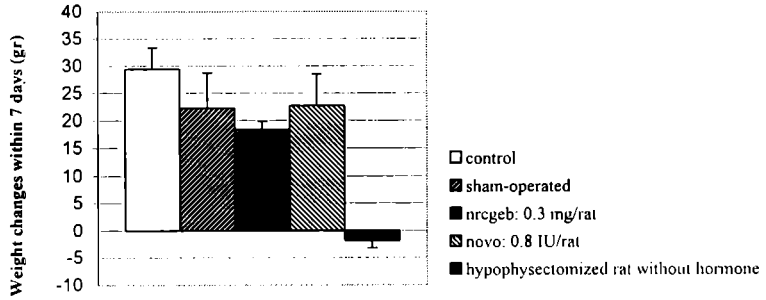

Figure 1. *Comparison between the bioactivities of an hGH synthesized in NRCGEB (purified using PI) and Novo hGH in 7 days treatment*

In hypophysectomized rats that treated with PBS growth regression occurs (1.9±1.3g decrease in weight compared to 29.4 ±3.95g increase in intact control rats $P<0.05$). Tretment of other subgropus with either the NRCGEB or the Novo hGHs resulted in weight gain, which is not different significantly from sham-operated and intact control rats (18.4±1.5g in NRCGEB treated rats and 22.7±5.8g in other).

Lack of growth in hypophysectomozed rats, which did not receive any hGH treatment indicates the complete excision of the pituitary. On the other hand, normal growth in sham-operated rats, which had the stress of the operation without pituitary excision indicates that the stress of operation has no effect on results. Due to identical weight increase in hypophysectomized rats who received either NRCGEB or Novo GH, it can be concluded that the GH of the NRCGEB has an equal activity to those of the commercially available GH and therefore can be introduced as a good replacement of endogenous GH. However, further studies will be neccesary to use this recombinant GH in those who suffer from lack of the hormone.

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