



RAPID COMMUNICATION

Intraperitoneal administration of gonadotropin-releasing hormone-PE40 induces castration in male rats

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Abstract

AIM: To evaluate the long-term effects of gonadotropin-releasing hormone (GnRH)-based vaccine on levels of GnRH antibody and testosterone, and vaccine-induced immunocastration on sexual behavior of male rats.

METHODS: The rats were treated with GnRH-PE40 intraperitoneally every other day for 12 wk. GnRH antibody and testosterone level in rat blood were determined by ELISA and radioimmunoassay, respectively. Morphological changes in testes and sexual behavior of rats were evaluated.

RESULTS: GnRH-PE40 induced a high production in GnRH antibody, decreased the serum testosterone level, testis atrophy and sexual function in rats.

CONCLUSION: Intraperitoneal administration of GnRH-PE40 produces structural and functional castration of male rat reproductive system by inducing anti-GnRH antibody.

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Key words: Gonadotrophin; *Pseudomonas aeruginosa* exotoxin A; Sexual behavior; Testis atrophy

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INTRODUCTION

Gonadotropin releasing hormone (GnRH) is secreted by the hypothalamus that stimulates the anterior pituitary gland to release gonadotropins in mammals. GnRH has been widely used as a single medical agent^[1] or a conjugated compound with other macromolecules^[2]. Studies indicate that GnRH- based chimerical proteins, consisting of GnRH and toxins, such as *tetanus* toxoid, *diphtheria* toxoid and *pseudomonas aeruginosa* exotoxin A, target specifically at positive tumor cells of GnRH receptor and kill them efficiently^[3-7]. It was reported that some of the chimeric proteins can effectively control reproductive (prostate, breast, ovary and endometrium) and digestive neoplasms^[8-11]. However, since its application in this field, high GnRH antibody titer always develops along with the treatment, which often impedes the use of these compounds^[12,13]. Some authors even reported that the high antibody titer induced by chimeric proteins leads to testis atrophy by depleting immunological hormone^[14].

Male livestock are routinely castrated in most countries to prevent their unpleasant odour (known as boar taint), aggressive behavior and unplanned breeding. As we know, intact male animals have superior feed conversion and leaner carcasses than surgically castrated pigs^[15]. Therefore, the problem is how to concurrently maintain both the intact of animals and the high quality of meat. If the similar strategy of anti-tumor agents mentioned above is applied to contraceptive vaccine, the problem can be possibly solved. Currently, scientists are trying to develop a substitute for the traditional surgical castration. Many preparations based on this theory have been applied to laboratory animals or pets for their immunological castration^[16,17]. It has been demonstrated that immunocastration can improve the meat quality and increase growth performance^[18-20].

GnRH-PE40, one of the recombinant single-chain fusion proteins consisting of GnRH fused to a binding-defective form of *pseudomonas aeruginosa* exotoxin A (PE40), has been developed as a preparation with potential functions of immune castration in male reproductive system. We report here the long term usage of GnRH- based chimeric protein which substantially induces castration in male rat reproductive system.

MATERIALS AND METHODS

Reagents

GnRH-PE40 is a genetic engineering product consisting of PE and GnRH from our laboratory.

Animals

Rats (specific pathogen-free) of Wistar strain, weighing 180–200 g, bought from Animal Center of Military Academy of Medical Sciences (Beijing, PRC), were housed in plexiglass cages (5 per cage) at temperature of 22°C–26°C and humidity of 60% in a 12 h light/dark cycle with free access to food and water. The experimental protocol was approved by the Animal Research Committee of Jinan University.

Treatment procedure

Twenty male rats were randomly divided into treatment group and control group and received intraperitoneal injection of 150 µg/kg of GnRH-PE40 and saline sodium, respectively, every other day for 12 wk. The sexual behaviors of rats were evaluated 12 h after the last injection. The rats were sacrificed under pentobarbital anesthesia 24 h after the last injection. Blood was collected from the heart of comatose rats for hormone or antibody determination. Testes were taken out, weighed, and fixed for histopathological evaluation.

Determination of GnRH antibody by ELISA

A 96-well microtiter plate was coated with 50 µL of 10 µg/mL of GnRH in carbonate bicarbonate buffer (CBB, pH 9.6) overnight at 4°C. After blocked with 3% bovine serum albumin (BSA) in PBS for 1 h at 37°C, the plate was incubated with diluted sera (1:100 to 1:12800) from the rats in different groups in 0.05% Tween 20/PBS with 0.3% (w/v) BSA for 1 h at 37°C. After washing, antibody was detected using horseradish peroxidase (HRP) conjugated goat anti-rat-IgG (BD Pharmingen, San Jose, CA, USA) for 1 h at 37°C. Signals were developed using DAB + substrate (Zhongshan Company, Beijing PRC) and optical density was determined at 490 nm using a BIO-RAD model 550 plate reader. Each measurement of a sample was conducted in duplicate. An absorbance equal to or greater than the mean + 3SE of the control group was considered positive.

Measurement of testosterone

Testosterone level in rat blood was measured by radioimmunoassay using a coat-A-count total testosterone kit (Diagnostic Products Corporation, Los Angeles, USA) according to its manufacturer's instructions. Each measurement of a sample was conducted in duplicate.

Histopathological examination of testis

Testes were fixed in Bouin's solution overnight at 4°C, followed by embedding, sectioning, staining with haematoxylin and eosin, and finally examined histopathologically under light microscope.

Mating behavior test

Ovariectomy was performed for female rats under

Table 1 Anti-GnRH antibody titer, testosterone level and testis weight in rats of the control and treatment groups ($n = 10$)

	Median Ab titer	Testosterone (µg/mL)	Testis weight (g)
Control	1:100	20.3 ± 4.7	4.5 ± 0.9
Treatment	1:1600 ^b	4.7 ± 0.8 ^b	3.1 ± 1.1 ^b

^b $P < 0.01$ vs control group.

ethyl ether anesthesia and 15 µg of estradiol benzoate was subcutaneously injected followed by 500 µg of progesterone 48 h later. Only those exhibiting a good sexual receptivity of male rats, that is, lordosis in response to mounting and with no reject behavior, were used.

The mating behavior of male rats was evaluated during the dark cycle in a sound-proof room, under a dim red light, according to the standard procedure^[21]. After a 10-min adaptation period in a rectangular glass observation cage (60 cm × 50 cm × 40 cm), a stimulus female rat was introduced to a male rat by dropping it gently into the cage. Then following behavioral parameters were recorded or calculated: latency of mount, intromission or ejaculation, and number of mounts, intromissions or ejaculations in a 30-min observation period.

Statistical analysis

Data were expressed as mean ± SE. Where analysis of variance indicated significant differences between groups with ANOVA, for the preplanned comparison of interest, Student's *t* test was applied utilizing the SPSS11.0 version. $P < 0.05$ was considered statistically significant.

RESULTS

GnRH-PE40 induced production of GnRH antibody

ELISA analysis showed that GnRH-PE40 induced a high production of IgG antibody to GnRH in rats. All the animals treated with GnRH-PE40 showed a positive anti-GnRH response. The antibody titers ranged from 1:800 to 1:3200 with a median of 1:1600 (Table 1).

GnRH-PE40 reduced testosterone in rats

Intraperitoneal administration of GnRH-PE40 provoked a significant decrease in blood testosterone of rats. The average testosterone level in rats treated with GnRH-PE40 was 5.4 µg/mL compared with 20.3 µg/mL in control group as shown in Table 1 ($P < 0.01$).

GnRH-PE40 resulted in testis atrophy

Histopathological examination showed that GnRH resulted in remarkable atrophy of testis in rats. The testis weight of rats treated with 150 µg/kg of GnRH-PE40 was decreased by 1.45-fold ($P < 0.01$) (Table 1). The seminiferous epithelium became thinner and the number of spermatogenic cells was decreased compared with that of control group (Figure 1).

GnRH-PE40 enhanced mating performance of male rats

Mating behavior test showed that GnRH-PE40 could improve mating performance of male rats. Compared

Table 2 Influence of GnRH-PE40 intraperitoneal injection on sexual behavior of male rats ($n = 10$, mean \pm SD)

	Latency(s) of			Number		
	Mount	Intromission	Ejaculation	Mount	Intromission	Ejaculation
Control	31 \pm 10	64 \pm 21	605 \pm 107	41 \pm 13	27 \pm 5	2.4 \pm 0.6
Treatment	105 \pm 43 ^b	300 \pm 27 ^b	> 1800	20 \pm 6 ^b	8 \pm 3 ^b	0

^b $P < 0.01$ vs control group.

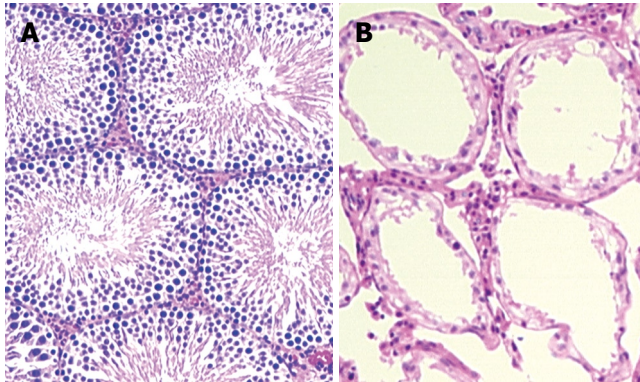


Figure 1 Influence of GnRH-PE40 intraperitoneal injection on spermatogenic cells. **A:** Testes from control rats demonstrating normal seminiferous epithelium (HE, $\times 400$); **B:** Testes from rats treated with 150 μ g/kg GnRH-PE40 demonstrating a significant decrease in spermatogenic cells (HE, $\times 400$).

with the controls, rats that received 150 μ g/kg of GnRH-PE40 exhibited an increased mount and an intromission latency, but a reduced mount and an intromission frequency ($P < 0.01$). No successful ejaculation was observed in rats treated with GnRH-PE40, while the mean ejaculation number in controls was 2.4 in a 30-min observation period (Table 2).

DISCUSSION

GnRH, a short peptide in its natural form, has a poor immunogenicity. When conjugated to a large carrier, it is likely to become an antigen, inducing antibody response. BcePred is a web-based tool for predicting B cell epitope regions in the sequence of an antigen with physicochemical properties. Identified properties of B cell epitope include hydrophilicity, flexibility/mobility, accessibility, polarity, exposed surface and turns. Using this tool, we predicted GnRH motif of GnRH-PE40 with the above properties individually using the default thresholds suggested by software, and found a potential epitope with a higher polarity than the default threshold of 1.8 at GnRH motif^[22]. We hypothesized that this potential epitope was capable of eliciting antibody reaction, which was verified by antibody assay, suggesting that GnRH-PE40 induces a high titer of anti-GnRH antibodies in sera of rats. Hormone determination and histopathological examination showed that intraperitoneal administration of GnRH-PE40 could decrease testosterone level, testis weight and sexual function, which are all indications of testis atrophy^[23-25]. Production of GnRH antibody is possibly the cause for testis atrophy^[26]. It is well-known

that hypothalamus-pituitary-gonadal axis is involved in the control of normal reproductive cycle and maintaining of the structure and function of the reproductive system. Anti-GnRH antibodies reduce the concentration of serum GnRH, which triggers hormone cascades including a lower pituitary LH/FSH and testosterone release, thereby affecting the structure and function of sexual organs^[27]. A high production of anti-GnRH antibodies in serum blocks primarily the function of GnRH, leading to the impairment of sexual organs^[28,29]. Studies showed that anti-GnRH antibodies induce testis atrophy in various laboratory animals and pets immunized with GnRH conjugates^[16,17].

In another study, we showed that 12-wk intravenous administration of GnRH-PE40 to monkeys produced only a low titer of anti-GnRH antibodies, but no marked testis atrophy was found (results not shown here), providing further evidence that antibodies against GnRH are the reason for testis atrophy.

In conclusion, GnRH-PE40 administration produces immunological castration by inducing anti-GnRH antibodies produced in response to the stimulation of B cell epitope of GnRH motif. GnRH-PE40 can be used in animal sciences as a non-surgical castration substitution for surgical castration^[30,31].

COMMENTS

Background

Surgical castration has been widely used as a routine way to prevent unpleasant odour and aggressive behavior of animals. Compared with surgical castration, immunocastration has more advantages such as easy operation in large scale, meat quality improvement and good animal welfare. Therefore, it is necessary to develop new drugs for animal immunocastration.

Research frontiers

Scientists in institutions or pharmaceutical companies are engaged in developing new drugs for animal immunocastration.

Innovations and breakthroughs

A new compound with potential use as an immunocastration agent was found and its physiological properties and actions were studied.

Applications

If the compound reported in this article can be used as an animal immunocastration agent, it will contribute greatly to farmers and production of high quality meat.

Terminology

Castration means surgical removal or artificial destruction of gonads. Immunocastration refers to castration methods based on immunological processes and techniques, such as use of castration vaccines.

Peer review

In the present study, the authors described the treatment of rats with GnRH-PE40,

which resulted in the production of antibodies against GnRH, lower testosterone levels and sexual behavior. The study was well designed and its results strongly support that GnRH can be used as a potential castration agent.

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