Original Article

Plasmid DNA vaccine coding eight repeats of gonadotrophin-releasing hormone induced atrophy of prostate in male mice

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A B S T R A C T

Background: Prostate hyperplasia and neoplasia are major illness of men and elderly dogs. Treatment of prostate cancer requires androgen deprivation surgery or therapy to prevent metastases and alleviate pain. Recently, six DNA vaccines have entered clinical trials against prostate cancer in humans with limited success. There is a need for new therapies that delay the establishment of malignancy and prolong survival.
Materials and methods: A plasmid DNA vaccine coding for eight gonadotrophin-releasing hormone (GnRH-I) interspersed in eight T-helper epitopes was used. Sexually mature male mice were immunized with the vaccine in hemagglutinating virus of Japanese envelope vector and boosted in nonionized surfactant vesicles in study weeks 0, 3, 6, 9, and 12. Plasma anti-GnRH-I antibody response, serum testosterone concentration, and effect on prostate were evaluated.
Results: Results of an indirect enzyme linked immunosorbent assay (ELISA) showed anti-GnRH-I antibody response (OD value) detected in the study week 3 (0.613 ± 0.179) with a highest response in the week 12 (1.205 ± 0.219). Serum testosterone concentration (ng/ml) in vaccinated mice was significantly reduced ($P > 0.000$, 0.761 ± 0.531) in the study week 24 in contrast to control serum (7.583 ± 1.251). Group average gross combined weight of prostate and seminal vesicles of vaccinated mice was significantly ($P < 0.000$) reduced in the study week 24 (319.75 ± 89.19 mg) in contrast to control weight (563.25 ± 108.60 mg). Sections of prostate stained with Goldner's trichrome showed profuse pink color secretion in control tubules, which however was absent in the vaccinated prostate. The lining epithelium of the vaccinated prostate was atrophied and did not enfold in its lumen.
Conclusions: Immunization strategy designed with the plasmid DNA vaccine in hemagglutinating virus of Japanese envelope and nonionized surfactant vesicles can be the genetic immunization platform. This vaccine bears potentials in terms of reducing serum testosterone concentration and induction of atrophy of prostate. Targeted ablation of native GnRH-I by genetic immunization could offer leverage to vaccinologists, seeking therapeutic target to control and prevent malignancy of prostate.

1. Introduction

The prostate is a male accessory sex gland, tubuloalveolar in nature and has got a profound role in mammalian reproduction. This gland secretes slightly alkaline fluid, milky or whitish in appearance and contains some smooth muscles that help expel semen during ejaculation[1]. The prostatic fluid expelled in the ejaculate provides better motility, longer survival, and better protection of the genetic materials of sperm[2]. However, prostatomegaly is a medical condition in which the prostate gland is abnormally large. The enlargement can be symmetrical or asymmetrical, painful or nonpainful. Prostatomegaly varies with age, body size, castration status, and breed and is a concern for health of pet animals and humans. Prostatomegaly is typically noted in middle-aged to older male dogs in and elderly people[3—5]. In the

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United States, prostate cancer (PC) is the most commonly diagnosed malignancy and is the second leading cause of cancer-related death among men. It is estimated that 220,800 new cases diagnosed annually and 27,540 deaths are caused by PC[6].

Historically, PC had been considered as "androgen-dependent" disorders as the gland contains androgen receptor (AR). Primary role of prostate is to respond to androgenic steroid hormones, such as testosterone and dihydrotestosterone. The PC remains highly dependent on the AR signaling axis in the affected glands[7]. The androgen secretion from the testes in mammals is regulated centrally by gonadotrophin-releasing hormone (GnRH-I)[8]; ablation of hypothalamic GnRH-I can be a potential tool to regress prostate[9].

It seems logical to use surgical and medical androgen deprivation therapy (ADT) to cure the patient or bring comfort of the elderly human patient. Surgical ADT is achieved through bilateral orchiectomy, whereas medical ADT may be achieved through the use of GnRH-I agonists or antagonists. Combined use of surgical and medical ADT has been shown to successfully palliate symptoms associated with metastatic PC[10]. Recent evidence suggests that the combined use of surgical and medical ADT is initially effective at stabilizing or causing disease regression in some patients; their effects are transient, and virtually all patients develop disease progression to a stage referred to as castration-resistant PC. Ultimately, metastatic PC remained incurable, and traditionally, patients therefore have to receive cytotoxic chemotherapeutics, such as mitoxantrone or docetaxel[11].

The standard management of PC since 1940s has been ADT by surgical or medical castration to suppress AR transcriptional activity[12]. Previously, we have engineered plasmid DNA vaccines[7,13] coding for four to five repeats of GnRH-I found to suppress gonadal androgen secretion. Male mice while immunized with the vaccines elicited anti-GnRH-I antibody response, reduced serum testosterone concentration, and induced atrophy of the prostate. This study used an improved version of GnRH-I vaccine that codes for eight repeats of GnRH-I. This vaccine while used in female mice found to induce higher level of anti-GnRH-I antibody response and suppressed fertility[14]. This study used male mice to elucidate the effect of GnRH-I immunoneutralization on the serum testosterone level and atrophy of the prostate. The mouse model is used in this study because it continues to be the most widely used animal model to study biological and pathological aspects of the prostate because of its small body size, ease in immunization and management, cost-effectiveness, and genome having approximately 95% similarity with human genome[15].

2. Materials and methods

2.1. Vaccine construct

The plasmid DNA vaccine[14] coding for eight repeats of GnRH-I and eight T-helper epitopes was used. The competent *Escherichia coli* M109 was transformed with the vaccine construct in LB Zeocin broth, and the plasmid DNA was extracted using a midi prep kit (Promega, USA). The DNA vaccine was diluted in nuclease-free water (1 mg/ml) and stored at −20°C until used.

2.2. Entrapment of plasmid DNA into hemagglutinating virus of Japanese envelope vector

GenomONETM-Neo EX is a nonviral reagent for transfection developed on the basis of the technology described by Kaneda *et al.* (2002)[16]. The plasmid DNA vaccine was incorporated into the hemagglutinating virus of Japanese envelope (HVJ-E) vector (HVJ envelope vector kit; Cosmo Bio Co., Ltd, Japan) in TE buffer at a concentration of 1 μg/μL. Briefly, ice-cooled TE buffer (260 μL) was

added to a tube containing freeze-dried 1AU HVJ-E (40 μL). The plasmid DNA vaccine in Tris EDTA (TE) solution (50 μg/50 μL) was added with the vector[14], mixed by pipetting, and it is freshly prepared each time before injected into the muscle of male mice.

2.3. Formulation of nonionized surfactant vesicles

Nonionized surfactant vesicles (NISV) were prepared with (11 mL) 136.4 mg 1-monopalmitoyl glycerol (Sigma−Aldrich Co Ltd, UK), 128.7 mg cholesterol (Sigma−Aldrich Co LtdUK), and 45.1 mg dicetyl phosphate (Sigma−Aldrich Co LtdUK) and were mixed in a 15 mL Pyrex test tube with the molar ratio of 5:4:1 and heated at 130°C in a dry-block calibrator(Grant Instruments, Cambridge, UK) until melted. Vesicles were formed when 2.5 mL of aqueous buffer (Phosphate buffered saline (PBS) pH 7.4) was added and the resulting suspension was vortexed vigorously for 1 minute. After shaking the suspension at 60°C for 2 hr, plasmid DNA vaccine (coding for GnRH-1) was added and entrapped by snap freezing the plasmid DNA and thawing the solution at 60°C five times. After a further 2 hr shaking at 60°C, entrapped DNA vaccine was centrifuged at 450,000 g for 45 minutes and was left on ice until immunization was carried out.

2.4. Immunization of mice

Five-week-old Swiss albino male mice ($n = 25$) was as purchased from the International Center for the Diarrheal Disease Research, Bangladesh (icddr,b). The mice were housed at a controlled condition in the Veterinary Clinic, Bangladesh Agricultural University, Mymensingh. Pelleted feed and water were supplied *ad libitum* and were replenished twice daily. Vitamin C was given at 20 mg/mouse daily with water. At the age of week 7, the animals were randomized and caged into groups of five and were ear coded. Mice were manually controlled before injecting plasmid DNA vaccine in the study week 0. A total of 50 μg of plasmid DNA vaccine in HVJ-E was injected into the right anterior quadriceps muscle. Primary immunization of male mice ($n = 25$) was carried out in subcutaneous tissue with the vaccine in HVJ-E vector (Group 1). The subsequent boosting was carried out in study weeks 3, 6, 9, and 12 with 50 μg of plasmid DNA vaccine/mice in NISV through intramuscular (i.m) route. Immunized mice were sacrificed in study weeks 3, 6, 9, 12, and 24 (5 mice in each point) to collect cardiac bleed, to measure serum testosterone level, and to evaluate the response of the prostate and seminal vesicles. Group 2 mice ($n = 05$) were maintained as untreated negative control for comparison.

2.5. Evaluation of anti-GnRH-I antibody response

An indirect ELISA was used to detect specific anti-GnRH-I antibody response (immunized mice sacrificed in the study week 24). In study weeks 0, 3, 6, 12, and 24, 100 μL tail bleeds were collected into heparinized capillary tubes (Selzer Labortechnik, Germany). Plasma was prepared by centrifugation at $200 \times g$ for 15 minutes and was stored at −20°C. The plasma anti-GnRH-I IgG response was measured in 96-well ELISA plates[7,13]. The A_{450} was read using a Multiscan ELISA plate reader (ELx800; BioTek Instruments, USA). A base line OD value 0.415 (twice the OD value of control reading) and above was considered positive anti-GnRH-I antibody response.

2.6. Determination of serum testosterone concentration

In study weeks 0, 3, 6, 12, and 24, the vaccinated (5 mice in each point) male mice were deeply sedated with ketamine hydrochloride (15 mg/kg body weight); cardiac bleeds were collected into the 1.5-mL centrifuge tubes, and the serum was separated by

centrifugation at 2,000 g for 10 minutes. Control mice (Group 2) were sacrificed in the study week 24, and cardiac bleed was collected in Eppendorf tubes. The serum testosterone level was measured using 125I-testosterone RIA test kit in direct quantitative radioimmunoassay (Beijing North Institute of Biological Technology, China). The radioactivity of the sediments was counted for 60s using a gamma counter (PC-RIA-MAS, Stratec Biomedical Systems, Germany). A standard curve was drawn using MS-DOS PC-RIA STD, Version 5.28, from the known standards of testosterone (0.1, 0.5, 2.0, 8.0, and 20 ng/mL). The serum testosterone concentration was measured by comparing the count of radioactivity against the standard curve of known testosterone standard. The group average serum testosterone concentration (ng/mL ± SD) was measured in each occasion.

2.7. Evaluation of effect on body weight and accessory sex glands

The body weight gain was measured in study weeks 0, 3, 6, 12, and 24 using a digital weighing machine. At terminal sacrifice (study week 24), the prostate and seminal vesicles were examined *in situ* and were dissected together to measure weight. The anterior prostate and body of seminal vesicle glands were fixed in 10% (v/v) buffered neutral formalin and were stained with hematoxylin and eosin and Goldner's trichrome staining[7]. The tissues were examined under a microscope at 10× and 40× magnification to identify changing morphology of glandular epithelium, volume of stored secretion, and fibromuscular reaction, if any.

2.8. Statistical analyses

Continuous data between vaccinated and control groups were compared with a Kruskal–Wallis unpaired χ^2 test using the Statistical Package for Social Sciences (SPSS), version 19.0 (SPSS, Chicago, IL, USA). A difference was considered significant at the $P < 0.05$ level.

3. Results

This is a follow up investigation of our previous study where the effect of GnRH-I immunoneutralization was studied in terms of inducing atrophy of the prostate in mice. This study used a plasmid DNA vaccine coding eight-GnRH-I repeats and applied new immunization strategy to enhance the level and duration of anti-GnRH-I antibody response.

3.1. Anti-GnRH-I antibody response and serum testosterone concentration

Results of immunization (Fig. 1) showed an early anti-GnRH-I antibody response (OD value) in the study week 3 (0.613 ± 0.179), reached its peak in the week 12 (1.205 ± 0.219) and showed a declining tendency in the study week 24 (0.817 ± 0.119). Group 2 mice did not develop anti-GnRH-I antibody response at any time point (0.252 ± 0.093). Mice vaccinated with NISV showed immune response detectable in the week 6 of immunization but induced persistently higher response in the study week 24. Serum testosterone concentration (ng/ml) in the vaccinated mice started declining following 6 weeks of immunization (5.351 ± 1.173) and very low level of serum testosterone concentration (0.761 ± 0.531) was seen in the study week 24 compared to unaffected serum testosterone concentration value (7.583 ± 1.251) in control mice (Fig. 1). Results of indirect ELISA and testosterone radioimmunoassay showed an intraassay variation of 7.5–8.6%. All the samples were run at the same time to avoid interassay variation. The serum testosterone concentration (0.486.41 ± 77.11) was significantly

($P < 0.003$) lowered in the study week 15 than control value (2.471.26 ± 568.00).

3.2. Effect on body weight gain and accessory sex glands

All the mice tolerated well the intramuscular and subcutaneous injections of plasmid DNA vaccine and did not suffer from agitation or fever. There was insignificant difference in body weight gain of the vaccinated and control males at the study week 0 (31.00 ± 0.81 g) and by the end of this study (50.50 ± 1.29 g). The gross combined weight of the prostate and seminal vesicles (Table 1) was significantly ($P > 0.001$) reduced in the study week 12 (439.75 ± 46.66 mg) and 24 (319.75 ± 89.19 mg) compared with the unchanged weight in control mice (563.25 ± 108.60 mg). To evaluate further the component of the glands really affected, detailed histopathology was carried out. Goldner's trichrome staining distinctly differentiated the components (tubular lumen, tubular epithelium and intertubular space) of the glands affected. A reduction of the stored secretion (brick/bright red color) in the tubular lumen (Fig. 2b) and atrophy of the lining epithelium of the prostate (Fig. 2b and d) were seen. There was apical atrophy of the lining epithelium of the prostate gland (cell becomes shorter) and reduction of the number of lining epithelium and its stored granules. The highest rate of atrophy of the prostate was seen in vaccinated mice in the study week 24 than in the prostate obtained from immunized mice in study weeks 3, 6, and 12. There was stromal reaction in the atrophied prostate of vaccinated mice in the study week 24. Seminal vesicles obtained from vaccinated mice in the study week 24 showed a reduction in its stored secretions and atrophy of glandular epithelium compared with the secretion rich alveoli in control seminal vesicles. Stromal reaction in the seminal vesicle of vaccinated mice was not seen.
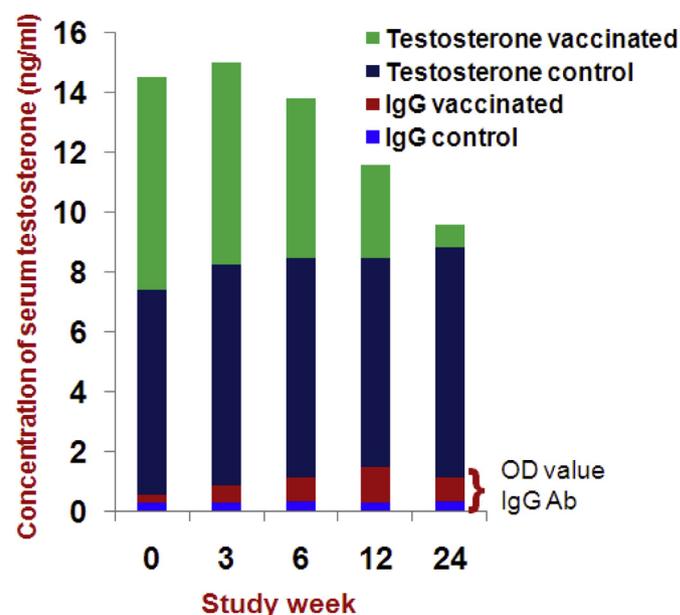

Fig. 1. The figure shows group average anti-GnRH-I antibody response (OD value, IgG Ab) and serum testosterone concentration (green and blue bars) in male mice as detected by ELISA and radioimmunoassay, respectively. Immunization of mice showed an early anti-GnRH-I antibody response in the study week 3 (0.613 ± 0.179), sharply rose the response until the study week 12 (1.205 ± 0.219, $P < 0.001$), and then, showed a declining tendency (0.817 ± 0.119) in the study week 24. Serum testosterone concentration (ng/ml) in vaccinated mice was significantly reduced ($P < 0.005$) after 6 weeks of immunization (5.351 ± 1.173), and very low level of serum testosterone concentration (0.761 ± 0.531, $P < 0.000$) was seen in the study week 24 compared with the unaffected serum testosterone concentration level (7.583 ± 1.251) in control mice.

Table 1
Immunization did not reflect any effect onto body weight gain. In study weeks 12 and 24, there was significant (*$P < 0.01$ and **$P < 0.001$, respectively) reduction in the combined weight of the prostate and seminal vesicle of vaccinated mice. There was also significant reduction of % weight of the prostate and SV in the Group 1 mice in study weeks 12 (*$P < 0.05$) and 24 (**$P < 0.001$). The values expressed were group average ± SD

| Criteria analyzed (group average)	Mice immunized with the vaccine ($n = 20$, Group 1)				Control mice ($n = 5$, Group 2)
	Week 3	Week 6	Week 12	Week 24	Week 24
Body weight (BW) in g	36.00 ± 0.81	40.25 ± 1.25	46.72 ± 1.23	50.50 ± 1.29	51.13 ± 2.3
Combined weight of the prostate and seminal vesicle (SV) in mg	427.43 ± 37.11	457.39 ± 49.1	438.85 ± 28.51*	319.75 ± 89.19**	563.25 ± 108.60
% of combined weight of the prostate and SV (per 100 g BW of mice)	1.186 ± 0.21	1.136 ± 0.17	0.939 ± 0.29*	0.751 ± 0.16**	1.101 ± 0.31
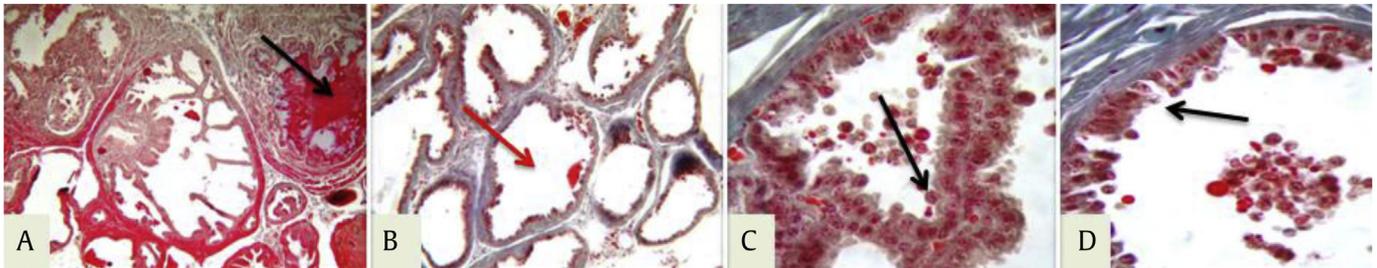
Fig. 2. Sections of the control (A and C) and vaccinated (B and D) prostate gland obtained in the study week 24 and stained with Goldner's trichrome. The stored secretion in tubules of the glands (A, arrow, 10×) appeared bright pink in the control section, which however was scanty in the vaccinated prostate (B, arrow, 10×). The lining cells of the control prostate appeared taller and the cells were enfolded in the glandular lumen (C, arrow, 40×). The lining epithelial cells of the vaccinated prostate appeared shorter (D, arrow, 40×), lower in densities and did not enfold in its lumen.

4. Discussion

Immunoneutralization of GnRH-I has been described as one of the effective mean to reduce serum testosterone level[7] and impaired reproductive functions without removing the reproductive organs.[17] This study used a plasmid DNA vaccine coding multiple T-helper epitopes into the vaccine construct to selectively induce Th-2 response. A histidine tag was incorporated in to the vaccine especially to extract fusion protein from translation sites. To achieve an early but higher immune response combination of vectors was used in immunization strategy. This study mostly focused on anti-GnRH-I antibody response, serum testosterone levels and effect of immunization on accessory sex glands.

4.1. Anti-GnRH-I antibody response and serum testosterone concentration

Results of indirect ELISA showed a sound increment of anti-GnRH-I antibody response in vaccinated mice in the week 12 (1.205 ± 0.219) and week 24 (0.817 ± 0.119) of immunization compared with the lack of response in group 2 control mice (0.252 ± 0.093). Previously, immunization trial was carried out in female mice with the vaccine in HVJ-E and boosted in PBS showed an early anti-GnRH-I antibody response (3 weeks). Primary immunization and subsequent boosts were carried out in female mice with the vaccine in NISV showed a delay in immune (week 6) but induced persistent higher response up to the study week 24.[14] To achieve an early but higher immune response primary immunization in mice in this study was, therefore, carried out in HVJ-E, and subsequent boosts were carried out with NISV and yielded better response (Fig. 1). Although there was a declining tendency of anti-GnRH-I antibody response in the study week 24, very low level of serum testosterone concentration was seen at this time point. This may be due to fact that the vaccinated mice possess anti-GnRH-I antibody response (0.817 ± 0.119) in the study week 24 is sufficiently higher to neutralize native GnRH-I and suppress gonadal function. This study measured only serum

IgG antibody response against GnRH-I; some other classes of antibody may be generated against genetic immunization that may have neutralized native GnRH-I and caused a decline in serum testosterone level.

In previous study we have used a plasmid DNA vaccine[13] coding five GnRH-I repeat, and mice immunized with the vaccine in HVJE showed plasma IgG anti-GnRH-I antibody response (0.63 ± 0.14) in the study week 15; the serum testosterone concentration (0.486.41 ± 77.11) was significantly ($P < 0.003$) lower in the study week 15 compared to control value (2.471.26 ± 568.00). There were differences in the serum testosterone concentration of the vaccinated mice of the previous[13] and present study. This may be due to the fact that the serum testosterone in the previous study was extracted by using diethyl ether and tested in enzyme immunoassay, and this study used serum sample in radioimmunoassay. However, the ratio of serum testosterone concentration between the vaccinated and control mice in the previous[13] (486.41/ 2471.26, about five fold reduction) and present (761/ 7583, about 10-fold reduction) study showed a significant improvement. In this study, a super secretory (fusion protein) plasmid DNA vaccine was used.[14] The vaccine and new immunization strategy applied in this study appeared to contribute constantly higher and relatively longer response; hence, there was 10-fold reduction of serum testosterone concentration. However, as the final boost of male mice with the vaccine was given in the study week 12 and showed declining tendency of serum anti-GnRH-I antibody response in the study week 24, further boosts with the vaccine in NISV at an interval of 12 weeks may maintain the anti-GnRH-I antibody response (study in progress). This immunization strategy may be a unique DNA vaccine delivery platform to achieve higher and longer immune responses, neutralization of native GnRH-I, and suppression of serum testosterone levels.

4.2. Effect on body weight gain and accessory sex glands

The male mice immunized with the vaccine through intramuscular and subcutaneous routes did not have effect body weight

gain. There was atrophy of the vaccinated prostate and seminal vesicles. The histopathologic variation of the vaccinated and control prostate and seminal vesicles were evaluated by staining thin sections using H&E and Goldner's trichrome staining. Goldner's trichrome staining distinctly differentiated the tubular lumen, tubular epithelium and intertubular space of the glands affected. Both the qualitative (reduce size of the cells) and quantitative (reduced densities of cells) atrophy of the glandular epithelium of prostate was seen leading to the reduction of stored granules in the epithelial lining of the prostate and its glandular lumen. The highest rate of atrophy of the prostate was seen in vaccinated mice in the study week 24 than prostate obtained from the immunized mice in study weeks 3, 6, and 12, a relation between higher level of anti-GnRH-1 antibody response and atrophy of the prostate was seen. The stromal reaction in the atrophied prostate of vaccinated mice in the study week 24 as seen could be due to reduction of the stored secretion, increasing inter lobular space; which in turn may promote fibrous connective tissues proliferation. Seminal vesicles obtained from vaccinated mice in study week 24 also showed a reduction in its stored secretions and atrophy of glandular epithelium but stromal reaction was not seen. Immunoneutralization of GnRH-I found to cause atrophy of the prostate at higher rate compared with the moderate level of atrophy of the seminal vesicles.

Prostatic enlargement, hyperplasia, and neoplasia are the commonest disorders in large population of men. A study was carried out at Alfort Veterinary Hospital, France to clarify and help veterinarians to manage the incidence of prostatic disorders.[18] During this investigation, a total of 72,300 male dogs (coming mainly from the Ile-de-France region) were registered in the Alfort Veterinary College database, and 481 of them (0.7%) were found to have prostatic disorder. Among these disorder most frequently recorded disorders were benign prostatic hyperplasia (45.9%), prostatitis (38.5%), abscesses (7.7%), cysts (5.0%), neoplasia (2.6%), and squamous metaplasia (0.2%). Canine prostatic hyperplasia and neoplasia are the commonly occurring malady in the USA, they are locally aggressive with a high rate of metastasis. Common metastatic sites reported were lymph nodes, lungs, liver, spleen, and bone.[19] The dog is the only large mammal, besides humans, that commonly develops spontaneous PC. Canine PC features many similarities with its human counterpart.[20] However, the incidence of PC is much lower in dogs and the precise cell of origin is not known in most cases.[21]

Prostatic enlargement and neoplasia in humans are the global health concern. In the USA, PC is a commonly diagnosed malignancy in men, and the second leading cause of cancer-related death.[22] The majority of newly diagnosed, organ confined prostate tumors are cured with surgery and-or radiation therapy, but historically one third of patients succumbed to recurrent illness. Ultimately, with a period of 8 years illness, the tumor spread from the primary sites and settled predominantly in the axial skeleton or pelvic/retroperitoneal lymph nodes.[23] For recurrent PC, ADT is commonly used[15] because the important stimulant for the growth of cancer cells is testosterone.[6] Testicle is the site of testosterone production, and adrenal cortex also produced significant amount of testosterone.[24] The prostate gland possesses receptors for testosterone and its growth is stimulated by testosterone whatever may be the sources.[25] There are studies describing local GnRH-I and its receptor mRNA expression in extra-hypothalamic-pituitary sites including the prostate.[26] GnRH-I affects multiple tissues not directly associated with the reproductive axis and got putative nonreproductive sites of action on the adrenal gland.[27] GnRH-I receptor mRNA have been reported in the mammalian adrenal and administration of a GnRH-I analog induced morphological changes in the adrenal cortex; these effects persisted after castration.[28] There has

been widespread attempt of ADT in men with the PC by using castration or GnRH-I agonist, which has been associated with improvements of overall therapy. However, the ADT is unable to sterilize the tumor growth even after castration.[29] This may be due to the fact that extragonadal testosterone, especially adrenal testosterone, provides stimulus for the growth of cancer cells. In light of these potential limitations of conventional ADT, GnRH-I immunoneutralization strategy may bring tremendous help by shutting down gonadal and adrenal stimulant for testosterone production by native GnRH-I.

There are peptides, protein, and DNA vaccines that have the potentials to reduce serum testosterone level and may contribute to ADT. To the best of our knowledge, six DNA vaccines are currently in the clinical trial to combat PC in humans. These vaccines were coded for human prostatic acid phosphatase (pTVG-HP), programmed death 1 receptor, ligand-binding domain of the AR (pTVG-AR, MVI-118), prostate-specific antigen and prostate-specific membrane antigen.[15,30] These vaccines offer significant advantages over other antitumor treatment approaches in terms of simplicity, manufacturing, and the absence of potentially health risk-like infectious vaccine but yet to meet the level of satisfaction. The plasmid DNA vaccine we have engineered or its fusion protein can meet the demand of GnRH-I immunoneutralization and reduce serum testosterone level by 10-fold compared with the control serum testosterone.

5. Conclusions

We have designed genetic immunization strategy using the plasmid DNA vaccine coding eight repeats of GnRH-I and eight T-helper epitopes. Primary immunization of mice with the vaccine in HVJ-E and boosts in NISV induced an early, constantly higher, and longer immune response. The plasmid DNA vaccine significantly reduced serum testosterone concentration and regress prostate in mice model. Targeted ablation of native GnRH-I by genetic immunization could offer leverage to vaccinologists seeking therapeutic target to combat or prevent malignancy of prostate in men and animals.

Conflicts of interest

The authors have no affiliations with or involvement in any organization or entity with any financial and nonfinancial interest in the subject matter or materials stated in this manuscript. However, the corresponding author preserved the right of the vaccine construct.

Acknowledgment

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.prnil.2018.01.001.

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