Theriogenology 86 (2016) 1589–1598

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effectiveness of an immunocastration vaccine formulation to reduce the gonadal function in female and male mice by Th1/Th2 immune response

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ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 17 May 2016
Accepted 18 May 2016

Keywords:
Immunocastration
GnRH
Vaccine
Chitosan

ABSTRACT

Immunocastration has emerged as an alternative to surgical castration in different animal species. This study examined the effectiveness of a new vaccine formulation for immunocastration using the biopolymer chitosan as adjuvant. First, female and male mice (n = 4), in three subsequent experiments were vaccinated at Days 1 and 30 of the study, to determine the immune response profile and gonadal alterations due to immunization. The results demonstrated that the vaccine was able to elicit strong antibody responses against native GnRH hormone ($P < 0.01$), with a T helper (Th) 1/Th2 immune response profile. Along with this, a suppression of gonadal activity with a decrease of luteal bodies (1.08 ± 0.22 and 4.08 ± 0.39) and antral follicles (1.17 ± 0.32 and 4.5 ± 0.38) in the ovaries of immunized females and control, respectively, and a reduction of seminiferous tubules size (142.3 ± 5.58 mm and 198.0 ± 6.11 mm) and germinal cellular layers (3.58 ± 0.26 and 5.08 ± 0.29) of immunized males and control animals, respectively, were observed ($P < 0.01$). Then, in a study of long-term immune response due to vaccination in female and male mice (n = 4) from two subsequent experiments, a suppression of gonadal function and an induction of a Th1/Th2 immune response was also observed, determined by both, immunoglobulin and cytokine profiles, which lasted until the end of the study (7 months; $P < 0.01$). The findings of this study have demonstrated that vaccination with a new immunocastration vaccine inducing a Th1/Th2 immune response against GnRH ($P < 0.01$) elicit a decrease of gonadal function in male and female mice ($P < 0.01$). Owing to long-term duration of the antibody levels generated, this vaccine formulation appears as a promising alternative for immunocastration of several animal species where long-lasting reproductive block is needed.

1. Introduction

Nonsurgical sterilization methods are highly desirable in domestic animals' population control [1] and sexual behavior control of male animals reared for meat production [2,3].

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http://dx.doi.org/10.1016/j.theriogenology.2016.05.019

Vaccines are an alternative for these issues, inducing immunologic block of GnRH. Gonadotropin-releasing hormone is a hypothalamic decapeptide that provides a primary drive for the reproductive axis, being present in male and female mammals. Active immunization against GnRH creates an immunologic barrier between the hypothalamus and the anterior pituitary gland, which prevents GnRH receptor-binding on pituitary gonadotropes, resulting in the suppression of gonadotropin secretion, inhibiting gametogenesis, and steroids production, as well as reproductive behavior. This strategy is commonly called immunocastration [4].

In male dogs immunized with fusion proteins including canine GnRH conjugated to tetanus toxoid [5] or T-helper cell epitope p35 originated from canine distemper virus F protein [6], elevated levels of GnRH-specific antibody in blood and reduced spermatogenesis were observed. In adult male cats, a single dose of GnRH vaccine based on keyhole limpet hemocyanin as carrier protein in a mycobacterial and oil emulsion as adjuvant, effectively blocked testosterone production and spermatogenesis in a short-term study [7]. In pigs, the use of a synthetic analogue of GnRH coupled to a tetanus toxin fragment as carrier protein, reduced boar taint without affecting other meat quality variables [8] and avoided unwanted aggressive and sexual behaviors, compared with unvaccinated control male pigs [9]. Immunocastration has also been investigated in wildlife species. In female bison, it was described that a single dose of GnRH vaccine, including keyhole limpet hemocyanin as carrier protein and a mycobacterial oil adjuvant, was effective preventing pregnancy for at least 1 year [10]. Although these immunocastration vaccines have been effective blocking reproductive activity in different models, most of them induced a short-lived effect [1] and in some, adverse effects associated to formulation components, such as persistent granulomas on the inoculation site, have been reported [11]. In addition, most of the developed vaccines against GnRH were based on the incorporation of a carrier protein in their formulation, and it has been described that the use of carrier proteins induces hapten immunosuppression [12,13]. For these reasons, study and development of new immunocastration vaccine formulations become necessary.

A recombinant peptide with a tandem repeat primary structure has been designed, expressed, and purified in the Laboratory of Veterinary Vaccines of the University of Chile incorporating the aminoacidic sequence of GnRH hormone fused to a nonpathogen-associated linker sequence, which does not need a carrier protein but an adequate adjuvant to enhance its immunogenicity. The immune response against an immunogen is determined by several factors, including adjuvant, antigen dose, route of administration, and host genetic factors [14]. One way of studying the response profile induced by any adjuvant is to determine the antibody subclasses and relate them to T helper (Th) involvement [15–17]. Biopolymer chitosan is one of the promising adjuvants used in recent years, which has demonstrated to be effective and able to induce a Th1/Th2 response in studies conducted by different research groups, including our own [18–20]. In a previous study developed in our laboratory, it was confirmed that chitosan is effective when it is used as an adjuvant with an immunocastration antigen in male rats [21].

The present study has determined the effectiveness of a new recombinant immunocastration vaccine against GnRH using low-molecular-weight chitosan as adjuvant to induce specific Th1 and Th2 immunoglobulin G (IgG) and cytokine immune response (Th1/Th2) associated with histological gonadal alterations in mice.

2. Materials and methods

2.1. Recombinant antigen purification through inclusion bodies

The recombinant antigen GnRXG/Q was designed as a tandem repeat of GnRH amino sequence flanked by a spacer region of 14 aminoacids to improve the immunogenicity of GnRH sequence in the whole peptide. Nucleotide sequence of 765 pb which encodes GnRXG/Q peptide GPPFSGGGGPPFSAQHWSYGLRPG repeated 10 times was synthesized with codon optimization of *Escherichia coli*, and subcloned in pJEXPRESS expression vector by DNA 2.0, which had a T5 promoter, kanamycin resistance, and a histidine tag for subsequent identification of the protein. The vector was transformed in *E coli* BL21 (DE3)plysS (Stratagen) for its expression in inclusion bodies.

Bacteria were grown in Luria broth enriched with yeast extract medium (tryptone 1%, NaCl 1%, and yeast extract 2%) supplemented with antibiotics, at 37 °C until A600 = 2. The expression of the recombinant peptide was initiated with 1-mM IPTG (Isopropyl β-D-1-thiogalactopyranoside) and orbital agitation for 18 hours at 37 °C. Bacteria were collected from the culture by centrifugation at 3000 × *g* for 10 minutes at 4 °C and resuspended in "inclusion bodies wash buffer I" (20-mM imidazole, 20-mM sodium phosphate, pH 7.4, 500-mM NaCl) for 1 hour at 37 °C. Then, cells were lyzed by two freezing cycles at −80 °C and thawing at 37 °C. DNA was sheared by a brief sonication, and inclusion bodies were recovered by centrifugation at 2000 × *g* for 20 minutes at 4 °C. Pellet was resuspended in "inclusion bodies wash buffer II" (0.5% [v:v] Triton X-100, 20-mM imidazole, 20-mM sodium phosphate, pH 7.4, and 500-mM NaCl). After another sonication and centrifugation, the pellet was solubilized in inclusion bodies solubilization buffer (8-M urea, 20-mM imidazole, 20-mM sodium phosphate, pH 7.4, 500-mM NaCl) and sonicated at 200 W for 3 minutes in 10-second pulses.

Soluble proteins were recovered from the supernatant after centrifugation (13,000 × *g* for 30 minutes at 4 °C), and the supernatant was filtered with a 0.45-μm filter, and then with a 0.22 μm. Subsequently, proteins were identified by protein polyacrylamide-SDS gel (12.5%) and transferred to a nitrocellulose membrane for immunoblot analysis by HisDetector Nickel-HRP kit (ThermoScientific). The protein concentration was estimated by bicinchoninic acid method (Calbiochem, Merk), according to the manufacturer instructions.

2.2. Chitosan as adjuvant

Low-molecular-weight chitosan (MW = 70 KDa, ≥75% deacetylation, 20,000 cps viscosity) (Sigma–Aldrich Corp)