Research Article
The Suitable Androgen-Deficient Models for Investigation of Eurycoma Longifolia, A Pro-androgenic Agent.

Putri Ayu Jayusman

Department of Biomedical Science, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia

Correspondence should be addressed to Putri Ayu Jayusman, putri.ayujay@gmail.com

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ABSTRACT
There are several androgen deficiency models that can be used to study the effects of pro-androgenic agents such as Eurycoma longifolia (EL). Suitable model must be chosen so that the androgenic effects of the agents tested could be demonstrated. As an example, EL was postulated to produce androgenic effects by stimulating the testosterone release by the testes. This makes the orchidectomy rat model of androgen deficiency unsuitable because EL cannot act with the removal of both testes. This review discussed the advantages and limitations of several androgen deficiency models and attempted to recommend the best model for studies on EL.

Keywords: Androgen, Orchidectomy, Osteoporosis, Leutinizing Hormone

1. Overview of the HPA Axis
The hypothalomo-pituitary-adrenal (HPA) axis is a central control and regulatory system of an organism which connects the central nervous system (CNS) with the hormonal system [1]. Any environmental changes, whether internal or external that disturb the maintenance of homeostasis is defined as stress [2]. HPA axis is a well-established stress-responsive neuroendocrine system that is vital for supporting normal physiological function, adapting to increased demands and maintaining homeostasis after a challenge[1].

Reproduction is regulated by a neuroendocrine axis known as the HPG axis, which involve hypothalamus, anterior pituitary gland and gonads. HPG axis comprises of gonadotropin releasing hormone (GnRH) - expressing neurons that trigger the release of GnRH which in turn acts on the anterior pituitary gland to stimulate the release of gonadotropins, the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [3]. The interaction of GnRH with its cognate receptor is essential in reproductive regulation [4]. Normally, it is mediated by GnRH receptor expressed only on the membranes of pituitary gonadotropes. Hence, the density of GnRH receptor on gonadotropes determines their ability to respond to GnRH. It was documented that, regulation of GnRH receptor gene expression is influenced by various factors including gonadal steroid hormones, inhibin, activin and possibly the most important one is GnRH itself [4].

2. Androgen-deficient Rat Model
The role of androgen in skeletal regulation has been proven by numerous studies in human and rodents, demonstrating the use of chemical or surgical castration which leads to accelerated bone loss [5]. Studies have reported that the goal of androgen ablation is to identify
agents that will consistently achieve and maintain the lowest testosterone levels possible [6]. Serum testosterone level of 50 ng/dl, was considered to be the castrate level [7]. This review attempts to discuss the various androgen-deficiency models induced by surgical or chemical castration or occurs naturally in aged male rats.

2.1 Orchidectomy
Surgical removal of testes or orchietomy is traditionally the gold standard for androgen ablation [6]. Aged orchietomized rat is a commonly used model for studies on androgen-deficiency-related diseases. This model was adopted in studies on Eurycoma longifolia (EL) and its active component known as quassinoids. EL is a herb with pro-androgenic properties and earlier studies reported that EL possessed aphrodisiac and testosterone enhancing effects [8]. However, EL treatment failed to produce protection against the deleterious effects of orchietomy on bone loss [9]. Testes is the possible site of actions of EL to promote testosterone release from the Leydig cells [10]. Therefore, removal of testes might be the reason why EL failed to confer protection against bone loss in orchietomy model. EL acted by stimulating the synthesis of testosterone by testes, making the study of androgenic potential of EL using orchietomized rat model not effective.

2.2 Chemical Castration Model

2.2.1 Luteinizing Hormone Releasing Hormone (LHRH) Agonists
Chemical castration can be performed by the use of injectable GnRH/LHRH analogues [6]. LHRH agonists exert a non-pulsatile, constant stimulation to the anterior pituitary gland which would lead to reduction of LH and testosterone production. However, LH release is reported to increase transiently for up to 2 weeks after the initial dose. This is referred as the hormonal surge phase, as the GnRH receptors are initially bound and activated [6,11]. After the immediate LH surge phase, subsequent inhibitory phase occurs due to reduction of GnRH receptors on the gonadotrophic cell membrane. At this phase, the LH and FSH productions are down-regulated and testosterone level is inhibited [6,12]. The receptors remain bound to the agonist for a while as these agents were originally designed with higher affinity to the GnRH receptors and higher duration of action [12].

Previous studies have reported that quassinoids of EL stimulated testosterone synthesis and elevated LH and FSH via the HPG axis [10]. The use of LHRH agonist to induce androgen deficiency may compromise the effect of EL as the GnRH receptors on the gonadotrophic cells remain bound to the agonist. In addition, the post-receptor mechanisms induced by the agonist led to secretion of biologically inactive gonadotropins [13]. The mechanism of action of LHRH agonists could prevent the action of endogenous GnRH, thus interfering the action of EL extract.

2.2.2 Luteinizing Hormone Releasing Hormone (LHRH) Antagonists
In contrast to agonists, LHRH antagonists act by competitive binding to the pituitary GnRH receptors, thereby preventing the action of endogenous GnRH and consequent gonadotropins suppression [14,15]. Unlike the LHRH agonists, initial hyper stimulation and hormonal surge did not occur [6]. Single application of LHRH antagonist resulted in profound long-lasting inhibitory but reversible effect on the reproductive system of adult intact male rats [16]. In contrast to LHRH agonist, treatment with LHRH antagonist preserves the responsiveness of the pituitary gland. Down-regulation, desensitization and post-receptor mechanisms are not initiated as competitive blockade of GnRH receptors take place [12]. It was also reported that suppression of serum concentration of LH could be achieved within 8 hours of administration of LHRH antagonist [17], while serum FSH concentrations were only slightly affected [18]. The suppressive effect could be overridden by the administration of pulsatile native GnRH which may displace the antagonist from receptors [12].

LHRH antagonist is more effective than the agonist in providing the effect of androgen deficiency as there is no occurrence of hormonal surge after the initial dosage and it produces immediate and rapid gonadotropins suppression. Apart from the competition of LHRH antagonist with GnRH for specific receptors, the endogenous GnRH could still produce their action [19]. It is postulated that GnRH is available in reproductive tissues to provide paracrine or autocrine role [20]. Therefore, treatment with EL extracts can be beneficial to this androgen deficiency model due to the fact that LHRH antagonist preserves the responsiveness of the pituitary gland.

2.2.3 Androgen receptor antagonists / anti-androgens
The anti-androgen agents or androgen receptor (AR) antagonists such as flutamide inhibit responses to androgens from both the gonads and adrenals [21]. AR antagonist is also applied together with orchietomy procedure or LHRH analogues to achieve a complete androgen blockade [22]. A non-steroidal anti-androgen, flutamide, exhibits anti-androgenic activity by inhibiting androgen uptake and/or inhibition of nuclear binding of the androgens in the target tissues [23]. Flutamide-mediated androgen deficiency is also employed as an animal model for the study of male reproductive development and human disease such as osteoporosis [24,25].

Recently, it was reported that flutamide induced alterations on the pituitary-testis axis in rats by disrupting the testosterone feedback [25]. Treatment with Panax ginseng, which promoted fertility, was found to ameliorate the alterations-induced by flutamide due to its direct or indirect effect on the HPG-axis. Hence, it could be hypothesized that the treatment with EL to this androgen deficiency could give comparable effects as EL extract also acts via HPG-axis.
2.3 Aged-Rat Model

Aged-rat model is the most natural model for androgen deficiency and usually adopted with orchidectomy procedure to produce androgen deficiency-induced osteoporosis in rats. Clarke & Pampolo [26] reported that the synthesis and secretion of GnRH were altered during aging. It was demonstrated in a previous study that GnRH level decreased with aging in intact rats but the pituitary responsiveness to GnRH remained intact [27]. It was suggested that secondary hypogonadism in aged-rats is to some extent related to decreased GnRH gene expression rather than to decreased pituitary responsiveness to GnRH. It was also demonstrated in 24 months old aged rats that there was reduction in hypothalamic level of GnRH, reduction in the capacity of the pituitary to synthesize or release LH and reduction in the capacity of the testes to produce testosterone [28]. The age impairment in hypothalamic/pituitary regulation of reproduction functions appeared to be remediable [27]. The use of aged-rat is preferable as it provides the most natural androgen-deficiency model and is very cost-effective. Nevertheless, to get the supply of aged male rats up to 24 months old for a research could be somewhat inconvenient.

3. Discussion

Surgical castration is an accepted procedure to produce androgen deficiency in test animal. However, studies have shown that EL supplementation was unable to emulate testosterone action completely in orchietomized model. The use of chemical treatments that produce similar effects to surgical castration can be considered for androgen-deficient model. An obstacle to the adoption of the LHRH analogues or AR antagonists as an agent to produce androgen deficiency in male rats is that the mechanism of action of EL extract involved HPG-axis. However, there might be a possibility for EL to exert its effect in LHRH antagonist-induced androgen deficiency in male rats. This hypothesis is supported by evidence from a recent study that has reported the administration of androgenic herbs, *Panax ginseng* was able to ameliorate the effect of flutamide-mediated androgen deficiency via HPG-axis. Aged-rat (up to 24 months old) is considered as the most natural model for androgen deficiency but somewhat inconvenient in terms of sourcing.

4. Conclusion

Based on compiled literature, it can be concluded that LHRH antagonist or AR antagonist-induced androgen deficiency is the recommended model for studies on pro-androgenic agents such as EL extract.

References


