New tissue-selective androgens: perspectives in the treatment of androgen deficits

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GENERAL INTRODUCTION

The requirement of normally functioning testes for normal growth and development of reproductive organs and secondary sex characteristics in men was already known centuries ago. Since the 1930s, when the chemical structure of testosterone was elucidated, various androgen preparations have been developed and used for indications relating to testosterone deficiency. Most of the currently available preparations are oral, injectable, or transdermal formulations of natural testosterone or testosterone-esters. However, due to the low potency of testosterone, low solubility of testosterone-esters, and low bioavailability after oral administration, these preparations have several disadvantages that relate to inconvenient routes of administration, frequency of administration and high doses needed. Attempts in the past to clinically develop potent synthetic androgens with a good safety profile have not been very successful [29]. In recent years, there is renewed interest in the physiology of testosterone and its potential therapeutic and contraceptive applications. Besides the classic indication of male hypogonadism, various conditions exist in which testosterone production is diminished (e.g. muscle wasting syndromes, osteoporosis, sexual dysfunction, the aging process) and replacement may be beneficial, both in men and women. This renewed interest initiated the design and development of new androgen preparations that are more potent, metabolically stable and tissue-selective than the currently available preparations, with the goal to improve therapeutic benefits and reduce risks and side effects. This chapter will describe factors that influence the biological activity of androgens, and the usefulness of tissue-selective androgen therapies in various therapeutic applications. For a better understanding of the mechanisms involved, first the normal physiology of testosterone is discussed as well as the currently available androgen preparations with its advantages and disadvantages.

ANDROGEN SECRETION AND METABOLISM

Testosterone is the principal circulating androgen. In men, it is secreted primarily by the Leydig cells in the testes at a daily production of about 6-7mg [24]. Testosterone regulates its own secretion via negative feedback by either inhibiting hypothalamic secretion of gonadotropin-releasing hormone (GnRH) or luteinizing hormone (LH) from the pituitary. Testosterone and follicle-stimulating hormone (FSH; produced in the pituitary) together are the essential hormones regulating the testicular sperm production. In men, the physiological range of circulating total testosterone levels varies between 10-35 nmol/L (≈ 3-10ng/mL). The major part of testosterone that circulates in the blood stream is bound to proteins, either tightly to sex hormone-binding globulin (SHBG) or more loosely to albumin, and only about 2% of circulating testosterone is unbound or ‘free’ [24]. Because of the rapid dissociation of testosterone from albumin, the non-SHBG bound fraction is considered the fraction that is available to the tissues (bioavailable testosterone).

Once the free testosterone has diffused into the target cell, it binds to the androgen receptor. The androgen receptor is a member of the steroid receptor family of nuclear transcription factors, whose action requires the binding of ligand [23]. The unliganded androgen receptor is a cytoplasmatic protein, and upon ligand binding receptor dimerization and nuclear translocation occurs. Other natural and synthetic compounds may also be capable of binding to the androgen receptor. Binding of ligand to the androgen receptor results in a conformational change to an active DNA-binding state. Binding of the active ligand-bound androgen receptor to a hormone response element, a regulatory DNA sequence in or near a target gene, alters the rate of transcription of the target gene, in concert with other transcription-regulating co-factors (both co-activators and co-repressors). This can result in either enhancing or repressing transcription [23]. Androgen receptors are present in many tissues in both men and women, indicating the diverse biological roles of androgens.
Testosterone has both direct and indirect actions. In addition to direct activation of the androgen receptor, testosterone has potent bioactive steroidal metabolites that amplify and diversify testosterone’s biological effects [24]. The two major metabolites of testosterone are 17β-estradiol formed by the enzyme aromatase (e.g. in bone, adipose tissue, brain), and 5α-dihydrotestosterone (DHT), a more potent androgen locally formed in target tissues by the enzyme 5α-reductase (e.g. in prostate and skin). Since the binding affinity of DHT to the androgen receptor is about 5 times higher than that of testosterone, the action of testosterone is potentiated in these tissues. In the circulation, DHT binds with greater affinity to SHBG compared to testosterone. In tissues that lack 5α-reductase (such as muscle), testosterone itself is the active androgen. The overall action of testosterone reflects the integrated response of each tissue to testosterone, DHT and estradiol.

The biological activity of androgens is modulated by their binding to specific plasma proteins, their overall metabolic clearance, tissue-selective metabolic transformation and activation of the androgen receptor.

CURRENTLY AVAILABLE ANDROGEN PREPARATIONS

In the 1930s, the chemical structure of testosterone was elucidated, enabling chemical synthesis of this hormone. As a substitute, natural testosterone is the first choice. However, testosterone is an androgen with a low potency and is metabolically unstable. Oral administration of natural testosterone did not have any effects due to inactivation of the compound in the liver and intestine. Therefore, ways to protect the molecule from premature metabolism had to be developed [20]. Early attempts to develop a suitable orally active androgen made use of 17α-alkylation, which makes androgens and steroids in general more resistant to metabolism. Alkylation at the 17α-position with an ethinyl group has been the solution for preparing metabolically stable, orally active estrogens and progestagens. However, 17α-ethynyl modifications of androgens resulted in lower androgenic potencies and co-currently higher progestagenic activity [29]. Only 17α-methylation resulted in a metabolically stable androgen, with selectivity for the androgen receptor. Besides the relatively low efficacy achieved with those molecules, oral administration of these preparations (e.g. 17α-methyltestosterone and fluoxymesterone) can cause hepatotoxicity in the doses needed to achieve sufficient androgenic effect in men (up to 50-100 mg per day). As a result, 17α-methyltestosterone and fluoxymesterone have become obsolete in men [20]. Low doses of methyltestosterone (1.25 mg or 2.5 mg) in combination with estrogens are still being used in female hormone therapy. Another attempt to circumvent the metabolic instability of testosterone resulted in the development of esters of testosterone, which act as pro-drugs. In the systemic circulation, these testosterone-esters are hydrolyzed by esterases, thereby releasing testosterone which will activate androgen receptors in target organs. The undecanoate ester of testosterone formulated in an oily solution in a soft gelatin capsule is the only available oral preparation of the natural hormone testosterone. It is designed to deliver testosterone to the systemic circulation via absorption through the intestinal lymphatic route, thereby circumventing first-pass inactivation by the liver [4, 10]. This oral formulation, with a long-term clinical experience, is easy to administer and allows flexible dosing. However, due to the limited potency of testosterone and the limited bioavailability of orally administered testosterone undecanoate, relative high doses of this product (120-160 mg per day) are required for optimal androgen treatment.

Intra-muscular injection of testosterone-esters in oil results in a depot of this pro-hormone, from which testosterone-esters are slowly released [3]. For intra-muscular application, several testosterone-esters (testosterone enanthate, testosterone propionate, testosterone cypionate) are widely used. However, these testosterone-ester injections can be painful and often result in an unfavorable pharmacokinetic profile in men. Intramuscular injections of testosterone-enanthate (100 mg every week or 250 mg every 2 – 3 weeks) produce high, even supraphysiological plasma levels of testosterone for a few days, followed by a gradual decline [3]. As a result, patients may experience side effects resulting from the supraphysiological testosterone plasma levels during the first few days after injection, such as mood disturbances and unfavorable changes in hematocrit and plasma lipid profile. Moreover, towards the end of the administration interval, plasma testosterone levels often drop below the normal range, which may result in the recurrence of hypogonadal symptoms. The high initial peak levels of testosterone after intramuscular injection seem a lesser problem with long-acting testosterone undecanoate injections [3, 6]. However, due to the limited potency of testosterone and low solubility of these testosterone-esters, large amounts of these esters are required in a large volume (about 4-8mL intramuscularly, depending on the vehicle), which limit the acceptability of this androgen therapy. Testosterone can also be delivered successfully from slow-release, subcutaneous pellets, which last for 4 – 5 months. However, the implantation of these testosterone pellets requires a small surgical procedure and the pellets are only available in a few countries.
Transdermal testosterone preparations (scrotal patches, transdermal patches, testosterone gel) are also effective in restoring testosterone to physiological levels. However, the delivery through the skin is not optimal and addition of skin enhancers such as ethanol are known to cause significant skin irritation [20]. Testosterone patches without skin enhancers are therefore large in size. Testosterone gel preparations also need to be administered to the skin in relatively large amounts in order to deliver enough testosterone to the systemic circulation (∼6-7mg testosterone per day). Although testosterone gel preparations are generally preferred over testosterone patches and injectables, the risk of transfer of hormones to others via skin contact is a disadvantage. In Europe, DHT is used for androgen replacement therapy as an alternative to testosterone, and a new DHT gel formulation is being tested in the US [28].

The clinical use of synthetic anabolic androgenic steroids such as nandrolone (19-nortestosterone) is largely restricted to the treatment of anemia and muscle wasting syndromes such as AIDS wasting.

SAFETY OF CURRENTLY AVAILABLE ANDROGEN PREPARATIONS

During effective testosterone substitution in hypogonadal men (by maintaining circulating testosterone within physiological levels), the occurrence of side effects is generally low. Inadequate dosing (too high or too low) is associated with more side effects. The current opinion among experts is that supraphysiological testosterone peaks should be avoided, as these might be especially related to the occurrence of side effects, such as fluid retention, overstimulation of erythropoiesis, adverse effects on lipid profile, and induction of gynecomasia. Another important concern is the potential effect of testosterone treatment on the prostate. The prostate is an androgen-sensitive organ, and testosterone treatment in hypogonadal men is known to induce prostate growth to a volume similar to eugonadal men. Although there is at present no evidence to indicate that long-term testosterone administration is associated with the development of prostate pathology (benign prostatic hyperplasia or prostate carcinoma), it is considered beneficial for prostate safety to avoid potentiation of new androgens via 5α-reduction.

From the above, it appears that many of the currently available therapies for androgen replacement (mainly in the form of injections or transdermal applications) have disadvantages. There is a need for androgens that are more potent and metabolically stable (thus requiring lower doses) and that are more tissue-selective in their action by inducing beneficial effects of androgen receptor activation (e.g. bone, muscle, brain) while reducing or eliminating the undesired side effects (e.g. overstimulation of the prostate and skin (causing acne), and the estrogenic side effects on the breast). In general, an oral product is still preferred since it is widely accepted by patients and prescribers, and combines ease of dosing and administration.

MODULATION OF BIOLOGICAL ACTIVITY OF STEROIDS

Structural modifications to a compound can lead to changes in its biological activity based on receptor binding affinity, differences in absorption, binding to plasma proteins and/or susceptibility to the action of metabolizing enzymes. For steroid hormones, this knowledge has resulted in various synthetic estrogens, progestagens and corticosteroids with a higher potency than the natural hormones. No significant progress has been made in developing new androgens with higher potency, that are metabolically stable and have a favorable safety profile. Although a marked increase in the androgenic potency was demonstrated for the 7α-methyl derivative of 19-nortestosterone (7α-methyl-19-nortestosterone; MENT) as early as 1960, no serious attempts were made to utilize these androgens due to their weak activity when given orally. In recent years, there was renewed interest in MENT by the parental route (subdermal implants). MENT is considerably more potent than testosterone due to its higher affinity for the androgen receptor. With the androgens 19-nortestosterone and MENT, which are not potentiated by 5α-reductase in the prostate, a prostate-sparing effect (less weight increase) is observed at a dose effective in suppressing serum LH and stimulating muscle growth in castrated rats [15]. These selective actions in different tissues may have therapeutic advantages.

Compounds with a tissue-selective estrogenic action such as tamoxifen, raloxifene and tibolone are clinically available for several indications in (postmenopausal) women. Various mechanisms may be responsible for the tissue-selectivity of these compounds [13, 18]. Tamoxifen and raloxifene are selective estrogen receptor modulators (SERMs), which are partially agonistic and antagonistic. SERMs act like estrogens in some target tissues but are antagonistic in other tissues. Apparently, the same ligand-receptor complex can exert different actions in different tissues. The underlying mechanisms have not been fully elucidated, but different interactions with various domains of the estrogen receptor and tissue-selective recruitment of transcription co-activators or co-repressors inducing cell-specific transcription may explain some of these tissue-selective effects of SERMs.
[11, 26]. Another compound with a tissue-selective action in postmenopausal women is tibolone. This synthetic steroid prevents bone loss and gives relief of climacteric complaints but does not stimulate the endometrium and the breast. In these tissue-selective effects of tibolone, steroid metabolizing pathways, enzyme regulation and receptor activation are involved [14]. The therapeutic advantages of modulation of estrogenic activity by these compounds has led to an interest in designing and developing compounds that modulate the androgenic response in a tissue-selective way. MENT is considered one of the first tissue-selective androgens, since it exerts androgenic activity in muscle and bone while having a lesser effect on the prostate [15, 27]. The basis for this tissue-selectivity is the absence of potentiation by 5α-reduction. To draw a parallel to the concept of SERMs, compounds with a tissue-selective androgenic response are often referred to in the literature as selective androgen receptor modulators (SARMs). However, we propose to reserve the acronym SARM for androgens with a partial agonistic/antagonistic profile like the classical SERM raloxifene. In the section below, the new developments in androgen research will be discussed.

**7α-METHYL-19-NORTESTOSTERONE (MENT)**

MENT is a synthetic androgen that is about 10 times more potent than testosterone. MENT is reported to be a substrate for aromatase, but not for 5α-reductase and thus its action on the prostate is not amplified which may have benefits in clinical use [17, 27]. Thus, a dose of MENT sufficient to maintain normal muscle mass will not hyperstimulate the prostate. Other studies indicated that MENT does not bind to SHBG [16]. Various studies have been conducted to investigate the pharmacology of MENT, but only few data are presently available on the effects of MENT in humans. In a trial in 45 healthy men, one, two, or four MENT implants were inserted subdermally [21]. The treatment induced a dose-dependent decrease in serum testosterone and gonadotropin levels (via negative feedback). All parameters returned to normal following removal of the implants after 4 weeks of treatment. The suppressive effect of MENT on spermatogenesis was studied in 34 healthy men [5]. These men were randomly assigned to one of three dose groups (one, two, or four MENT implants) for 180 days. No serious side effects or signs of androgen deficiency were reported and the data showed acceptable efficacy on suppression of spermatogenesis in the four-implants group, indicating that MENT may be an attractive compound in the development of a hormonal male contraceptive. In 20 hypogonadal men, the ability of two MENT implants to support sexual behavior was compared with that of intramuscular injections of testosterone-enanthate (200mg at 3 week intervals) [1]. The study used a crossover design and each treatment phase had a duration of 6 weeks. Responses to treatment were measured by a combination of daily diaries, questionnaires and interviews. The results showed that the response to two MENT implants was similar to that of standard treatment with testosterone-enanthate. The implants were well tolerated and preferred over the frequent injection of testosterone-enanthate. A recent abstract reported on the effects of 24 weeks treatment with one or two MENT implants on prostate volume, bone mineral density and muscle mass in 16 hypogonadal men [2]. Prior to receiving MENT, these hypogonadal men had all been treated with testosterone-enanthate injections for 24 weeks. The data during MENT treatment showed a decrease in prostate volume while muscle mass increased, which seems consistent with a tissue-selective effect of MENT. Data on bone mineral density showed varying effects per site (lumbar spine and hip) and dose. However, the small sample size and unblinded design of the study hinders interpretation of these data. More data are needed to further evaluate the clinical effects and long-term safety of MENT.

**TISSUE-SELECTIVE, NON-STEROIDAL ANDROGENS**

Recently, non-steroidal molecules have been identified that are selective for the androgen receptor [19]. Non-steroidal androgens can neither be potentiated upon 5α-reduction nor aromatized to estrogenic compounds. LG121071, an orally active androgen agonist, showed binding affinity to the androgen receptor and was effective at suppressing castration-induced elevation of LH in rats [7, 9]. The effects of LGD2226 have been investigated in skeletally mature orchiectomized (ORX) male rats as an animal model of male hypogonadism. LGD2226 was able to prevent bone loss and maintain bone quality in ORX rats by stimulating bone formation, while also inhibiting bone turnover. LGD2226 also exerted anabolic activity on the levator ani muscle in rats [25]. Recently, abstracts were presented on androxolutamide (GTx-007), an orally active, tissue-selective, non-steroidal androgen reported to possess strong anabolic activity and little androgenic activity in animal models [8, 12, 22]. However, the development of these tissue-selective, non-steroidal androgens is in an early stage, and no information is yet available on the effects of these new androgens in humans.
CLINICAL USE OF NEW, TISSUE-SELECTIVE ANDROGENS

Possible indications of tissue-selective steroidal and non-steroidal androgens focus on the treatment of androgen-related diseases and disorders, such as (late-onset) hypogonadism, osteoporosis, sexual dysfunction and wasting syndromes, both in men and women. Another interesting application is replacing testosterone in male contraception. Currently, the most effective male contraceptive regime is a combination of an androgen with a progestagen. Administration of a progestagen results in a dose-dependent suppression of pituitary gonadotropins and consequently, a decrease in testosterone levels and a reversible inhibition of spermatogenesis. An exogenous androgen is required to compensate for the reduced testosterone levels. It has further been suggested that compounds with a tissue-selective modulation of androgen action could provide opportunities in the prevention and treatment of prostate cancer, benign prostatic hyperplasia, acne and hirsutism via exhibiting antagonistic effects in the prostate and skin, and agonistic effects in other tissues. However, much remains to be done in evaluating the various potential actions of new, selective androgens on tissues and to assess their clinical efficacy and safety.

DEVELOPMENTS WITHIN ORGANON

Organon has a long history in androgen research and development. With its expertise in this field, Organon is now working on the design and development of new, tissue-selective androgens, both steroidal and non-steroidal, for use in various therapeutic applications. The development of new steroidal androgens within Organon’s research program is focusing on selective androgens with a high potency, oral activity, metabolic stability, favorable pharmacokinetics allowing once daily application, no increased androgen receptor activation after α-reduction, some mild aromatization because some estrogen activity might be needed in males (e.g. on bone and central nervous system), and a favorable overall safety profile. Non-steroidal androgens are considered favorable with respect to the safety profile. In the further development of these non-steroidal compounds, attention should be paid to the impact of the lack of metabolism of these compounds (e.g. no aromatization to estrogens), the effects on the prostate and the long-term safety of this treatment especially because hypogonadal men will be treated life-long.

Recent research activities have resulted in the identification of several interesting androgenic compounds for the indications male hypogonadism and male contraception. The first compound is currently being investigated in early Phase I clinical research, and the second compound will enter the early clinical phase soon. Pharmacological evaluation shows that both compounds have a higher affinity for the human androgen receptor and, are metabolically more stable than testosterone, resulting in good oral efficacy, and have a prostate-sparing effect. These pharmacological observations in animal models provide a promising basis for the further clinical development of these compounds in humans.

CONCLUSION

The development of new, tissue-selective androgenic compounds is an exciting advance in androgen research. Androgenic compounds, either steroidal or non-steroidal, that induce beneficial effects upon androgen receptor activation while reducing or eliminating the undesired side effects (e.g. prostate, skin, breast) can offer many therapeutic benefits over currently available androgen therapies. However, much needs to be done to evaluate the clinical efficacy and long-term safety in humans.

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