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Characterisation of the pharmacological profile of desoxymethyltestosterone (Madol), a steroid misused for doping

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Abstract

Desoxymethyltestosterone (DMT), also known as Madol, is a steroid recently identified to be misused as a doping agent. Since, the knowledge of functions of this substance is rather limited, it was our aim to characterise the pharmacological profile of DMT and to identify potential adverse side effects. DMT was synthesised, its purity was confirmed and its biological activity was tested.

The potency of Madol (DMT) to transactivate androgen receptor (AR) dependent reporter gene expression was two times lower as compared to dihydrotestosterone (DHT). Receptor binding tests demonstrate that DMT binds with high selectivity to the AR, binding to the progesterone receptor (PR) was low. In vivo experiments in orchiectomised rats demonstrated that treatment with DMT resulted only in a stimulation of the weight of the levator ani muscle; the prostate and seminal vesicle weights remained unaffected. Like testosterone, administration of DMT resulted in a stimulation of IGF-1 and myostatin mRNA expression in the gastrocnemius muscle. In the prostate proliferation was stimulated by TP (testosteronepropionate), but remained unaffected by DMT. Remarkably, treatment with DMT, in contrast to TP, resulted in a significant increase of the heart weight. In the liver, DMT slightly stimulates the expression of the tyrosine aminotransferase gene (TAT).

Our results demonstrate that DMT is a potent AR agonist with an anabolic activity. Besides the levator ani weight, DMT also modulates the gene expression in the musculus gastrocnemius. The observed stimulation of TAT expression in the liver and the significant increase of the heart weight after DMT treatment can be taken as an indication for side effects. Summarizing these data it is obvious that DMT is a powerful anabolic steroid with selective androgen receptor modulators (SARM) like properties and some indications for toxic side effects. Therefore, there is a need for a strict control of a possible misuse.

Keywords: Desoxymethyltestosterone; Madol; Skeletal muscle; Anabolic steroid; Doping; Liver

1. Introduction

Anabolic-androgenic steroids (AAS) have been banned in sport since 1976. According to the worldwide doping control statistics they are the most frequently

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detected substances (WADA, 2004). Since the ban of AAS, athletes and their entourage have always tried to find new products and applications to evade the doping controls. Rumours of new steroids, produced and used specifically to escape doping tests in sport have been spread since more than 15 years. In 2002, Catlin et al. found a never-marketed steroid called norbolethone in two urine samples (Catlin et al., 2002). In 2003, a track and field coach sent a syringe with an oily substance to the United States Anti-Doping Agency. The substance proved to be tetrahydrogestrinone (THG) the first true "designer steroid", designed, synthesised and distributed solely as an undetectable doping agent (Catlin et al., 2004; Labrie et al., 2005; Friedel et al., 2006). Even more recently, a new "designer steroid", desoxymethyltestosterone (DMT) or "Madol" was discovered, and its detection in urine described (Sekera et al., 2005). DMT is not really a new steroid. A patent for the synthesis of Madol was awarded in 1961. DMT was first reported in the literature in 1963 (Kincl and Dorfman, 1964). However, DMT has never been approved for human use by the FDA and more importantly, no safety and efficacy data are available.

Therefore, the aim of our study was to further characterise the pharmacological profile of DMT and to identify potential adverse side effects. DMT was synthesised and its identity, purity and biological activity were tested. The binding affinity of DMT to the androgen receptor (AR), but also to the progesterone receptor (PR) was determined. To verify, if the binding of DMT to the respective receptors results in a biological activity in vivo, orchiectomised rats were treated for 10 days with equimolar doses of DMT and the reference compound testosteronepropionate (TP). Effects on prostate, seminal vesicle, heart, the levator ani muscle, and the gene expression in the musculus gastrocnemius and prostate were investigated and correlated to the regulation of the tyrosine aminotransferase gene expression (TAT) in the liver.

2. Materials and methods

2.1. Substances

Testosteroneproprionate (TP) was provided by the Institute of Biochemistry, German Sports University Cologne. Purity of the substances was verified by mass spectrometry. Dihydrotestosterone (DHT) was obtained by Sigma–Aldrich (Deisenhofen, Germany). R1881, aldosterone, dexametasone and progesterone were provided by Schering AG (Berlin, Germany).

2.2. Synthesis of DMT

DMT was prepared from 5α-androst-2-en-17-one by Grignard alkylation according to established procedures (Sekera et al., 2005). Briefly, 50 mg (0.18 mmol) of 5α-androst-2-en-17-one were dissolved in diethyl ether and added slowly to a solution of methylmagnesium bromide. The resulting mixture was refluxed, cooled to ambient temperature and diluted with a saturated solution of ammonium chloride. The resulting product was extracted with diethyl ether, and the organic layer was evaporated to dryness yielding 35 mg (0.12 mmol) of DMT (purity >90% as determined by GC–MS analysis).

2.3. Yeast reporter gene assay

For the assessment of androgenicity, a concentration dependent assay in the widely used androgen inducible yeast screen androgen receptor assay was performed (Sohoni and Sumpter, 1998). The yeast-based androgen receptor assay was cultured as previously described (Zierau et al., 2003). The yeast strain contained both a stably transfected androgen receptor (AR) construct and an expression plasmid carrying androgen-responsive sequences controlling the reporter gene lac-Z (encoding the enzyme β-galactosidase). Androgenic activity resulting from the enzymatic hydrolysis of chlorophenol red β-D-galactopyranoside was read at 540 nm using a colorimetric assay. In a concentration dependent analysis of reporter gene activity, the half maximal induction of β-galactosidase activity was a direct measure for the affinity of the compound to the AR, and, therefore, the androgenic activity could be estimated.

2.4. Receptor binding assay

Cytosols containing the relevant steroid hormone receptors were provided by Schering AG, Berlin. Serial dilutions of the investigated substances were incubated in the presence of 5 nM [3H]R1881 or [3H]Progesterone in 300 μl cytoslolic extract at 0–4 °C. After 16 h, 100 μl of the incubation mixture was pipetted into the assay tubes containing 500 μl hydroxilapathit slurry (60% in 50 mM Tris). After incubation for 20 min tubes were centrifuged for 2–3 min at 4 °C and 600 × *g*. Two millilitres of 50 mM Tris were added to each tube. The mixture was vortexed and centrifuge at 600 × *g* as above. The TRIS washing procedure was repeated three times. Following the last wash and decanting, 1.5 ml of ethanol was added to each tube and the tubes were centrifuge at 600 × *g* for 10 min. The supernatants were decanted into scintillation vials and after adding of scintillation cocktail samples were counted.

2.5. Hershberger assay

Male Wistar rats (130 g) were obtained from Janvier Laboratories (Le Genest St. Isle, France) and were maintained under controlled conditions of temperature (20 ± 1 °C, relative humidity 50–80%) and illumination (12 h light, 12 h dark). All rats had free access to standard rat diet (SSniff R10-Diet,