Androgenic properties of the dietary supplement 5α-hydroxy-laxogenin

Carolin Beer - Annekathrin M. Keiler

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Abstract
Dietary supplements sold for anabolic benefits or performance enhancement often contain substances, which are non-approved and might lack quality controls. With regard to athletes, the inclusion of substances or methods in the prohibited list of the World Anti-Doping Agency is based on medical or scientific evidence. 5α-hydroxy-laxogenin is a synthetic spirostane-type steroid, which is contained in dietary supplements and advertised as anabolic agent. To date, evidence is missing on anabolic or androgenic activity of 5α-hydroxy-laxogenin. We investigated its androgenic potential in two in vitro bioassays. While no activity was observed in the yeast androgen screen, 5α-hydroxy-laxogenin was able to trans-activate the androgen receptor in human prostate cells in a dose-dependent manner. Interestingly, a biphasic response was observed with antagonistic properties at lower concentrations and agonistic effects at higher concentrations tested. The demonstrated androgenic properties of the higher concentrations demonstrate that further investigations should focus on the safety as well as on potential anabolic effects of 5α-hydroxy-laxogenin. This is of interest with regard to abuse for doping purposes.

Keywords 5α-hydroxy-laxogenin · PC3(AR)2 cells · Yeast androgen screen · Androgen receptor · Dietary supplements

Introduction

The World Anti-Doping Agency (WADA) publishes annually the lists of substances and methods prohibited as doping (World Anti-Doping Agency 2022). Prerequisite for including a substance or method is the fulfillment of two out of three criteria defined by the WADA. Those are the medical or scientific evidences that a substance or method possesses either sport performance enhancing effects, (potential) health risk to athletes or violates the spirit of sport (World Anti-Doping Agency 2021).

Dietary supplements might contain illegally added compounds with performance enhancing effects, which, for instance, are published by the U.S. Food and Drug Administration on its Dietary Supplement Ingredient Advisory List (U.S. Food and Drug Administration 2021). Among them, 5α-hydroxy-laxogenin, a derivative of the spirostane-type steroid laxogenin is advertised as plant-based anabolic agent. Whereas the natural occurrence has been shown for laxogenin in several smilax species as well as in two Allium species (Akahori and Yasuda 1963; Kim et al. 1991; Kubo et al. 1992; Baba et al. 2000; Timité et al. 2013), there is no proof of a natural existence of 5α-hydroxy-laxogenin. In contrast, Avula et al. recently showed the synthetic origin of 5α-hydroxy-laxogenin detected in dietary supplements (Avula et al. 2019). Since 2019, 5α-hydroxy-laxogenin is included in the FDA list and proven to be contained in dietary supplements (Cohen et al. 2020), but data on potential anabolic effects are missing.

The intention of the present study was to get a first insight on potential androgenic properties of 5α-hydroxy-laxogenin. Therefore, androgen receptor transactivation was investigated in vitro in the yeast androgen screen as well as in a reporter gene assay in a human prostate cell line.

Materials and methods

Sigma-Aldrich (Munich, Germany) provided dihydrotestosterone (DHT, purity ≥ 97.5%), bicalutamide (purity ≥ 98%) and hydroxyflutamide (OHF, purity ≥ 98%).
5α-hydroxy-laxogenin (5α-OH-laxogenin, purity > 95%) was purchased from Biomol (Hamburg, Germany). Carl Roth (Karlsruhe, Germany) provided dimethyl sulfoxide (DMSO, purity ≥ 99.5%). All test compounds were dissolved in DMSO. Chlorophenol red-β-D-galactopyranoside was purchased by Roche (Mannheim, Germany). Dulbecco’s Modified Eagle’s Medium (DMEM/F12), fetal bovine serum (FBS) and penicillin/streptomycin (P/S) were supplied by BioWest (Nuaillé, France). Qiagen (Hilden, Germany) provided Attractene Transfection Reagent. G418 was supplied by Calbiochem, Merck (Darmstadt, Germany). Luciferase assay system was supplied by Promega (Mannheim, Germany). AppliChem (Darmstadt, Germany) provided bovine serum albumin fraction V (BSA). Dr. Aria Baniahmad (Institute for Human Genetics, University Hospital Jena) kindly provided human PC3(AR)2 cells and the reporter plasmid focusing on potential anabolic properties and safety of 5α-hydroxy-laxogenin intake are necessary.

Results and discussion

In the yeast androgen screen, DHT stimulated the reporter gene expression in a dose-dependent manner (Fig. 1a). However, none of the 5α-hydroxy-laxogenin concentrations induced the reporter gene β-galactosidase (Fig. 1b).

In contrast, 5α-hydroxy-laxogenin was able to induce the luciferase expression in human PC3(AR)2 cells in a biphasic dose-dependent manner, with antagonistic effects at lower doses (0.01–1 µg/mL) and agonistic effects at higher doses (Fig. 2). Co-incubation with bicalutamide, a non-steroidal AR antagonist, antagonized the 5α-hydroxy-laxogenin-induced luciferase activity (Fig. 2). This clearly demonstrated that 5α-hydroxy-laxogenin binds to the human AR and acts as an agonist in the PC3(AR)2 cells at higher doses. The discrepancy between the two bioassays might be due to the different co-factor pattern in yeast and mammalian cells as well as the additional yeast cell wall, which might prevent diffusion. Hence, false negative results in the yeast androgen screen of substances showing clear androgenic properties in mammalian cells are possible, e.g., shown for p,p’-DDE by Gaido and colleagues (Gaido et al. 1997; Endocrine Disruptor Screening and Testing Advisory Committee 1998).

The observed androgenic potential of higher 5α-hydroxy-laxogenin concentrations observed herein raises possible safety concerns regarding reproductive organs (e.g., prostate). Besides the androgenic activity, further investigations should focus on potential anabolic activities of 5α-hydroxy-laxogenin. The proof of anabolic effects would meet WADA’s criterion on performance enhancement. Moreover, data on pharmacokinetics as well as on biotransformation should be gathered to contribute to an estimation of the efficacy and safety of 5α-hydroxy-laxogenin.

In conclusion, we showed androgenic potential of 5α-hydroxy-laxogenin in an in vitro bioassay for the first time. As this synthetic spirostane-type steroid is marketed as dietary supplement for athletes, future investigations focusing on potential anabolic properties and safety of 5α-hydroxy-laxogenin intake are necessary.
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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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