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Prepubertal chrysin exposure upregulates either AR in male ventral prostate or AR and ERα in Skene's paraurethral gland of pubertal and adult gerbils

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ABSTRACT

Chrysin is a plant-derived polyphenol that has the potential to increase endogenous testosterone levels both by inhibiting the aromatase enzyme and by stimulating testicular steroidogenesis. The effects of chrysin on the prostate are unknown, especially during its development and functional maturation. Thus, the aim of this study was to evaluate the effects of chrysin prepubertal exposure on the male and female prostates of both pubertal and adult gerbils. To evaluate the possible androgenic responses of chrysin, gerbils were also exposed to testosterone. Male and female gerbils were exposed to chrysin or to testosterone cypionate from postnatal day 15 to 42. Male and female gerbils were euthanized at either 43 days or 90 days age. The prostates were collected for biometrical, morphological and immunohistochemical analysis. The results showed that prepubertal exposure to chrysin had differential effects on the prostate of both pubertal and adult animals. The prostates of male and female pubertal gerbils showed no histological alterations, although there was increased frequency of androgen receptor (AR) in males and females, and estrogen receptor alpha (ERα) in females. Adult males and females presented developed prostate glands, with higher cell proliferative rate. In addition, AR and ERα frequency remained high in the prostate of adult animals. These results demonstrated that prepubertal exposure to chrysin disrupts steroid receptors regulation in the prostate, potentiating the response of this gland to the biological effects of endogenous steroids. In this context, excessive consumption of phytoestrogens during the critical stages of development should be considered with caution.

1. Introduction

Chrysin (5, 7-dihydroxyflavone) is an active compound of the flavone class, naturally found in several plant foods, such as medicinal herbs, propolis, honey, chamomile, mushrooms and fruit bark [1,2]. This flavone has been considered beneficial for health due to its anti-inflammatory, antioxidant, anti-allergic, antiviral, anxiolytic and anticancerogenic properties [3,4].

Food supplements rich in chrysin are related to improving serum testosterone levels. Chrysin stimulates testicular steroidogenesis by upregulating the expression of StAR gene with concomitant increase of testosterone production [5,6]. Moreover, chrysin also boosts testosterone *via* inhibition of aromatase, an enzyme that converts testosterone to estradiol [7,8].

Based on its hormonal properties, chrysin has been employed for the treatment of reproductive disorders. Studies with rodents have demonstrated that chrysin causes a delay in testosterone decline during aging, increases either the libido or the quantity and motility of seminal sperms [3,5,9]. Indeed, due to its potential to inhibit the bioavailability of endogenous estrogens, chrysin has been suggested for gynecomastia,

Abbreviations: C, control group; Chr, chrysin group; T, testosterone group; AR, androgen receptor; ERα, estrogen receptor alpha; PCNA, proliferating cell nuclear antigen

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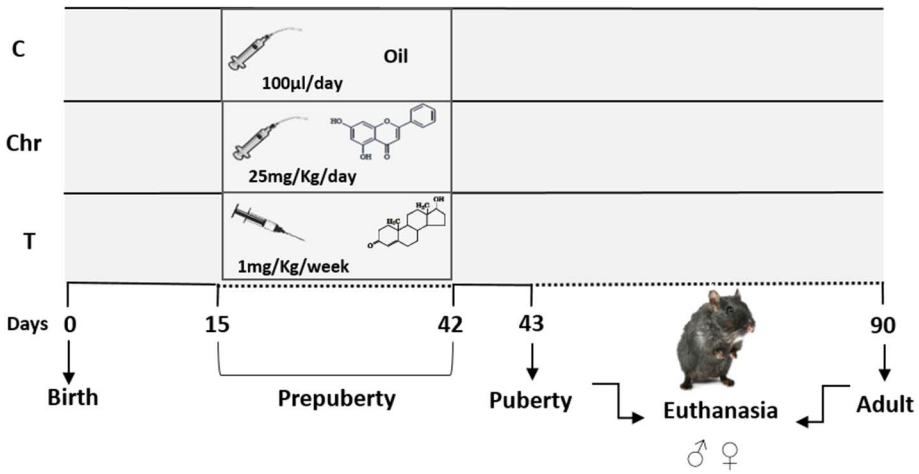

Fig. 1. Schematic representation of the experimental protocol employed in this study.

breast and prostate cancer treatment [10,11]. Despite its promising benefits, the studies regarding chrysin effects upon male and female reproductive systems are scarce. Thus, the effects of chrysin on the reproductive accessory glands, especially the prostate, are unknown.

The prostate is an accessory gland of the mammal reproductive system, and its function is to synthesize and to secrete an alkaline liquid that promotes nutrition and sperm survival [12]. Contrary to classical descriptions, the prostate is not a male-exclusive organ, since it can also be found in women and in several rodent species (also termed as Skene's paraurethral gland) [13,14]. Hormonal regulation and prostate functional identity acquirement are established either during intrauterine or postnatal development [15]. Thus, the early exposure to biologically natural compounds may change the intrinsic developmental prostate program, leading to permanent morphophysiological disorders.

Since chrysin is able to increase testosterone levels and also to decrease estrogen bioavailability, we hypothesize that intake of flavonoid-enriched foods may change the prostate morphophysiology, especially in critical phases of glandular development. Therefore, the purpose of this study was to evaluate the effects of chrysin prepubertal exposure on the male and female prostates of both pubertal and adult gerbils. Indeed, in order to investigate the androgenic potential of chrysin, we analyzed, comparatively and under the same experimental conditions, the effects of testosterone upon gerbil prostate.

2. Material and methods

2.1. Chemical procedures

The chrysin synthesis was performed in two steps, according to protocols described by Ramesh and co-authors, with modifications [16]. First, we have prepared chalcone intermediate by using Claisen-Schmidt condensation between trihydroxyacetophenone and benzaldehyde [17]. This reaction was performed in 60% sodium hydroxide in ethanol at room temperature for 24 h. The reaction mixture was poured into crushed ice and acidified with HCl at pH 3. The crude product was subject to liquid-liquid partition with ethyl acetate. The combined organic phases were concentrated under reduced pressure and chromatographed over silica gel, to furnish 2′,4′,6′-trihydroxychalcone in 44% yield. Second, the conversion from chalcone to flavone was achieved by intramolecular nucleophilic substitution [18]. Solution of 2′,4′,6′-trihydroxychalcone in glycerol and iodine was refluxed for 8 h. After complete conversion confirmed by TLC analysis, the reaction mixture was extracted with ethyl acetate by liquid-liquid partition. The crude product was purified over silica gel, yielding chrysin (23%). Chrysin was obtained as pale yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6): 12.82 (br s, 1H), 8.06 (d, 2H), 7.59 (m, 3H), 6.96 (s), 6.54 (d) and 6.23 (d). ^{13}C NMR (125 MHz, DMSO-d_6): 182.3, 164.9, 163.7, 161.9, 157.9, 132.5, 131.3, 129.6, 126.8, 106.6, 105.0, 99.5 and 94.6.

2.2. Animals

The male and female gerbils were maintained in the Histophysiology Laboratory of the Federal University of Goiás (UFG; Goiânia – GO) under controlled room temperature (25 °C) on a 12 h light/dark cycle. All animals were housed in new polyethylene cages and filtered water was provided from glass bottles. Gerbils were fed with standard rodent food *ad libitum* (Labina-Purina®; composition: 23% protein, 4% fat, 5% fiber and 12% minerals). Animal handling and experiments were performed according to the ethical guidelines of the Federal University of Goiás (CEUA/UFG, protocol n° 110/15), following the Guide for Care and Use of Laboratory Animals.

2.3. Experimental design

We used 30 adult female and 30 adult male gerbils (*Meriones unguiculatus*), all between 90 and 120 days old, for mating. We randomly matched one male to one female to form independent families. The mating day was determined by the presence of spermatozoa in the vaginal smears; this day was considered as day zero, being the initial day of the gestational period [19]. After birth, pups (48 male and 48 female) were destined to form the following groups: Control group (C) – animals received oral doses of the dilution vehicle (corn oil; 100 µL/animal) from the 15th until 42nd day of postnatal life (impubertal or prepubertal phase according to Pinto-Fochi et al. [20]; Chrysin group (Chr) – animals received oral doses of chrysin (25 mg/kg/day; according to the dose used by Darwish et al. [6]) from the 15th until the 42nd day of postnatal life; Testosterone group (T-positive control group) – animals received subcutaneous injections of testosterone cypionate (1 mg/kg/week; Deposteron/EMS) from the 15th until the 42nd day of postnatal life. Male and female gerbils were euthanized at either 43 days (puberty onset) or 90 days (adult onset) of postnatal life. In this way, 12 experimental subgroups were formed (Fig. 1). All animals (n = 8/subgroup) were euthanized by a lethal dosage of anesthesia (100 µL/100 g), which was prepared with a mixture (proportion of 1/1) of anesthetic (Cetamin, Syntec) and muscle relaxant (Xylazine, Vetbrands). The body, gonadal and whole prostatic complex (correspondent urethral segment, ventral, dorsolateral and dorsal prostate lobes in males; and vaginal segment, corresponding urethral segment and prostatic tissue in females) were weighed.

2.4. Light microscopy

The whole prostatic complex (n = 8 glands/group) were fixed by immersion in methacarn (proportions: methanol 60%, chloroform 30%,