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Nomegestrol acetate: steroid receptor transactivation profile in Chinese hamster ovary cells and ovulation inhibition in rat and monkey

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Abstract

Background: Activity of nomegestrol acetate (NOMAC), levonorgestrel (LNG), drospirenone (DRSP), dienogest (DNG) and progesterone on human steroid receptor transactivation was investigated. Ovulation inhibition by NOMAC, LNG and progesterone was tested.
Study Design: The progestogen receptor profile was determined in Chinese hamster ovary cells transfected with human progesterone B (PRB), androgen, estrogen (ER_α and ER_β), glucocorticoid (GR) and mineralocorticoid (MR) receptors, respectively. Ovulation inhibition was tested in rats and monkeys.
Results: Agonistic potency rankings for PRB were LNG=NOMAC≫progesterone≫DRSP>DNG. No antagonistic activity at PRB was observed. Only LNG had androgenic activity. Antiandrogenic potency rankings were LNG≫NOMAC>progesterone>DNG>DRSP. All agents were devoid of activity at ER_α, ER_β and GR. Only progesterone, DRSP and LNG had anti-MR activity. The NOMAC dose inhibiting ovulation at 50% ranged from 0.14 mg/kg (monkey) to 1.25 to 5.0 mg/kg (rat).
Conclusion: Nomegestrol acetate is a selective progestogen and a potent inhibitor of ovulation in the rat and monkey.

Keywords: Steroid hormone receptor; Ovulation; Progestogen; Nomegestrol acetate; Rat; *Macaca fascicularis*

1. Introduction

Nomegestrol acetate (NOMAC) is a 19-norprogesterone derivative with high binding affinity to the progesterone receptor (PR) [1]. Nomegestrol acetate has been used clinically alone as well as in combination with estradiol (E2) for hormone replacement therapy [2]. This progestogen has also been shown to be effective in inhibiting ovulation in fertile women [3–5] and is in development in combination with 17β-E2 as a monophasic combined oral contraceptive [2].

Progestogens have been characterized mainly by determining receptor binding affinity and biological activity. Studies of receptor binding and in vivo activity of NOMAC suggested that it acts as a relatively pure progestogen on the regulation of rat uterine progesterone and other receptors [1,6,7]. However, binding affinity alone does not allow the discrimination between the activation of agonists and antagonists [8,9].

Hormone-dependent transcription was studied using functional human steroid receptors stably transfected together with a luciferase gene into a Chinese hamster ovary (CHO) cell line [10]. Agonistic and antagonistic activity of NOMAC and other clinically relevant progestogens was determined at the human progesterone B (PRB), androgen (AR), estrogen (ER_α, ER_β), mineralocorticoid (MR) and glucocorticoid (GR) steroid receptors. The ability of NOMAC to inhibit ovulation in rats and monkeys was studied to confirm progestogen activity in vivo.

2. Materials and methods

2.1. Transactivation studies

To determine agonistic and antagonistic profiles of progestogens, cellular assays were performed in a similar manner to those published previously [10,11]. Chinese hamster ovary K1 cells were obtained from the American

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Type Culture Collection (ATCC, Manassas, VA, USA) and stably transfected with cDNAs encoding human PRB (hPRB), AR (hAR), MR (hMR), GR (hGR), ER_α (hER_α) and ER_β (hER_β). The cells were cotransfected with a mouse mammary tumor virus promoter luciferase reporter system for hPRB, hAR, hGR and hMR, and the rat oxytocin promoter luciferase reporter system for hER_α and hER_β [10–12]. For hAR and hMR cell lines, clonal selection resulted in minor expression of endogenous GR. In order to circumvent GR interference in the responses, GR activity was blocked by onapristone (0.3 μM) and mifepristone (1 μM) in the hAR and hMR cell lines, respectively. These cell lines were seeded at 5×10^3 cells/well in 384-well plates in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (Gibco, Life Technologies, Carlsbad, CA, USA) and Ham's F12 medium (Gibco), supplemented with 5% charcoal-treated defined bovine calf serum (Hyclone, ThermoFisher Scientific, Waltham, MA, USA). Steroid test and reference compounds were dissolved in dimethyl sulfoxide (DMSO) diluted in complete culture medium (0.1% final DMSO concentration in culture medium) and coincubated with the cells for 16 h at 37°C and a 5% CO_2 atmosphere. At the time of testing, plates were taken out of the incubator, and when they reached room temperature, 25 μL of Steadylite Plus (Perkin-Elmer, Boston, MA, USA) for cell lysis and luciferase measurement was added to all wells. After incubation for 1 h in the dark at room temperature, the plates were measured for luminescence with an EnVision multiple plate reader (Perkin-Elmer). Each measurement was recorded as the mean response of four independent plates tested in one assay.

2.2. Evaluation of responses

Maximal agonistic activity was determined for hPRB, hAR, hER_α, hER_β, hMR and hGR using the reference compounds Org 2058 (0.0003–10 nmol/L), a PR agonist; 5_α-dihydrotestosterone (0.0003–10 nmol/L), an AR agonist; E2 (for both hER_α, hER_β; 0.0003–10 nmol/L), an ER agonist; aldosterone (0.003–100 nmol/L), an MR agonist; dexamethasone (0.003–100 nmol/L), a GR agonist, respectively. Both reference and test compounds (NOMAC, levonorgestrel [LNG], drospirenone [DRSP], dienogest [DNG] and progesterone) were determined in 10-point dose–response curves with a dilution factor of 3.16 for each step. For each assay, efficacy of the reference compound was set at 100%. Test compounds with <80% of the reference activation were categorized as demonstrating partial activation of the receptor. The effective concentration at which 50% of the maximum effect was reached was defined as the EC50. Antagonistic activity was determined using the test compounds (NOMAC, LNG, DRSP, DNG and progesterone) to inhibit the activity of the agonistic reference compounds used for maximal activation. The response of the reference antagonists Org 31710 (0.003–100 nmol/L), a PR antagonist; hydroxy-flutamide (0.3–10,000 nmol/L), an AR antagonist; ICI 164.384 (0.03–1000 nmol/L), an ER antagonist; Org 34116 (0.3–10,000 nmol/L), a GR antagonist; and spironolactone (0.3–10,000 nmol/L), an MR antagonist, was compared to the test compounds. The concentration at which 50% of the maximum effect of the agonist was inhibited was defined as the IC50.

2.3. In vivo ovulation inhibition studies

2.3.1. Rats receiving a single subcutaneous dose

Mature female Wistar rats (approximately 200 g) were used. The rat estrous cycle was monitored by means of daily vaginal smears. When three consecutive, regular 4-day cycles had been observed, the animals in the metestrous phase received a single subcutaneous (SC) injection of NOMAC or progesterone, each at doses of 0.25, 1 and 4 mg/rat (n =5–6 rats per dose), or vehicle (n =17). The animals were sacrificed on the third day after the administration of the test compound, the oviducts were removed and the number of ova in the oviduct was microscopically assessed. Ovulation was evaluated by comparing the percentage of ovulating animals receiving NOMAC or progesterone with the percentage of ovulating animals receiving vehicle.

2.3.2. Rats receiving multiple daily oral doses

Mature female Wistar rats (approximately 200 g) were treated for one complete cycle (4 days, from estrus to proestrus). Nomegestrol acetate (n =7–8 rats per group) was administered orally in a dose range of 0.25–2.5 mg/rat per day; LNG (n =4–8 rats per group) was administered in a dose range of 0.25–10 mg/rat per day. The animals were sacrificed on the day after the last treatment, the oviducts were removed and the number of ova in the oviduct was microscopically assessed. Ovulation inhibition was evaluated by comparing the ovulation rate in NOMAC- and LNG-treated animals with the ovulation rate in vehicle-treated controls (n =11).

2.3.3. Cynomolgus monkeys (Macaca fascicularis) receiving multiple daily oral doses

The ovulation-inhibiting activity of NOMAC was also determined in 31 female cynomolgus monkeys. Animals were trained for oral daily drug administration, vaginal swabbing (for determination of menstrual bleeding) and blood sampling by femoral venipuncture. Animals with regular menstrual cycles (26–32 days) were entered into the study protocol.

After two control cycles, animals received oral NOMAC from cycle day 5 to 25 (0.1, 0.25 and 1 mg/kg per day; n = 10–11 for each dose). Animals were randomized to initial treatment groups using stratified randomization to balance the groups for body weight and cycle length. Day 1 of each menstrual cycle was defined as the first day that menstrual bleeding was detected. During each of the two control cycles and the one treatment cycle, blood samples were drawn three times per week during days 5 to 20 for determination of E2