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Non-clinical studies of progesterone

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Abstract

Progesterone is a steroid hormone that is essential for the regulation of reproductive function. Progesterone has been approved for several indications including the treatment of anovulatory menstrual cycles, assisted reproductive technology, contraception during lactation and, when combined with estrogen, for the prevention of endometrial hyperplasia in postmenopausal hormonal therapy. In addition to its role in reproduction, progesterone regulates a number of biologically distinct processes in other tissues, particularly in the nervous system.

This physiological hormone is poorly absorbed when administered in a crystalline form and is not active when given orally, unless in micronized form, or from different non-oral delivery systems that allow a more constant delivery rate. A limited number of preclinical studies have been conducted to document the toxicity, carcinogenicity and overall animal safety of progesterone delivered from different formulations, and these rather old studies showed no safety concern. More recently, it has been shown in animal experiments that progesterone, its metabolite allopregnanolone and structurally related progestins have positive effects on neuroregeneration and repair of brain damage, as well as myelin repair. These recent preclinical findings have the potential to accelerate therapeutic translation for multiple unmet neurological needs.

Keywords

Progesterone; pharmacodynamics; toxicology studies; carcinogenicity; neuroregeneration

Introduction

Progesterone plays an essential role in many aspects of reproduction, including ovulation, transformation of the endometrium, and pregnancy maintenance. The multiple and complex effects of progesterone on the female reproductive process are the result of its binding to and activation of progesterone receptors (PRs), including sub-units PR-A and PR-B[1–3] and membrane receptors[4,5].

Progesterone effects the secretory transformation of an estrogen-primed endometrium, a process which prepares the endometrium to receive and nourish a fertilized ovum. In addition, depending on the level of progesterone secretion and the duration of the luteal phase, progesterone can prevent the over-proliferation of endometrial tissue[6].

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The main indications for which progesterone has been approved include the treatment of anovulatory menstrual cycles, assisted reproductive technology (ART), and, when combined with estrogen, the prevention of endometrial hyperplasia in postmenopausal hormonal replacement therapy regimens[6–8].

A few products delivering the physiological hormone progesterone have been developed and approved for various indications including hormonal therapy. These include micronized progesterone given orally or via a vaginal insert, and products designed to deliver micronized progesterone directly from the vaginal mucosa: a vaginal ring[9], a vaginal gel[10] and a vaginal insert[11]. Micronized progesterone is identical to the naturally occurring hormone secreted by the ovary, placenta, and adrenal gland. Therefore, in contrast to new chemical entities, there were few preclinical studies conducted with these products[9–11].

Non-clinical pharmacology

Receptor binding

Progesterone has been shown to bind to progesterone receptors (PR-A and PR-B) in reproductive organs, including ovary, fallopian tubes, uterus, and mammary gland[1–3], as well as to PRs in the anterior pituitary and hypothalamus[12,13]. PR-A appears to be necessary for progesterone-dependent aspects of female fertility, while PR-B is involved in progesterone-related differentiation of mammary glandular tissue[3]. Nuclear PR has also been found in thymus, vascular smooth muscle, bone, and the peripheral nervous system[3,13] as well as in primate and human male reproductive organs, including the epididymis, prostate, and male mammary gland[14,15]. Membrane PRs have been found in the reproductive and non-reproductive tissues of rat and primate[4,5].

Pharmacodynamics

Pharmacokinetics (absorption, distribution, and excretion) determine how much of the steroid administered is available to tissues, primarily by measuring its blood level, and the amount that enters the cells is determined by the extent to which it is bound to carrier proteins that cannot cross the cell membranes. After progesterone enters the systemic circulation, it is distributed between blood and tissues by passive diffusion. The pattern of distribution is mainly regulated by its binding to transport proteins and tissue receptors. A relatively small amount of progesterone is bound with high affinity and low capacity to corticosteroid-binding globulin but not to sex hormone-binding globulin[6].

Primary pharmacodynamics—The primary pharmacodynamics of natural progesterone given by non-oral routes of administration has been evaluated in several animal species[16–18].

The effects of progesterone delivered via a vaginal ring on vaginal bleeding, ovarian function, and the endometrium were evaluated in the rhesus monkey[16]. In this study, 25 mature females were assigned to treatment with intravaginal rings delivering progesterone (235 or 1770 μg/day), norethisterone (39 or 340 μg/day), or placebo for 52 weeks. At end of treatment, suppression of ovarian function was observed in treated groups. The endometrium was atrophic in all monkeys in the high-dose progesterone and norethisterone groups[16].

The effect of progesterone delivered by an intrauterine system (IUS) on endometrial transformation and fertility was studied in the female rabbit[18]. An IUS delivering progesterone (10, 65, or 150 μg per day) was implanted in one uterine horn while the contralateral horn served as a control. The endometrium in both horns exhibited progestational effects in the 65- or 150-μg/day groups. Ovulation occurred in all subjects except those in the 65-μg/day group. Fertility returned quickly in all animals after IUS removal[18].

Secondary pharmacodynamics—Pregnant female rats were treated with progesterone (1.5 mg/kg subcutaneous) or vehicle control from day 8 to day 21 of gestation, a dose comparable to a human dose of 100 mg/day[19]. Prenatal exposure to progesterone had no significant effect on reproductive success, neonatal mortality, and physical maturation. Progesterone-treated offspring weighed less than control animals initially ($p < 0.05$), a difference that disappeared by days 37–38. Brain DNA content was significantly greater among progesterone-treated offspring ($p < 0.02$, Student's t-test). The only statistically significant behavioral difference detected was a reduction in mean head-lifting responses on the open field test among progesterone-treated offspring ($p < 0.01$, test for trend). It was concluded that prenatal exposure to progesterone had no consistent effects on the central nervous system in male rats[19].

Pharmacokinetics and bioavailability

The pharmacokinetics of exogenous progesterone has been explored in a few studies in animal models.

In female ovariectomized rats treated with subcutaneous capsules containing progesterone (20, 40, 110, or 220 mg of crystalline progesterone), serum levels increased and then declined, reaching a steady-state value from day 7 to day 24 after capsule insertion[17].

In dogs, progesterone bioavailability (relative to intravenous dosing) was significantly higher following administration of 25 mg of progesterone by vaginal tablet versus vaginal suppository[20]. In six female dogs, a single progesterone dose of 2 mg/kg, given by intramuscular injection, resulted in a progesterone mean (± standard deviation) serum C_{max} of 34.3 (± 7.8) ng/ml at 1.8 (± 0.2) h after dosing; serum progesterone declined to pre-administration values (0.9 (± 0.2) ng/ml) by 72 h after the dose[21].

Absorption, distribution, metabolism and excretion

In ovariectomized female rats, administration of a single dose of progesterone, 500 μg/kg intravenously, was followed by widespread distribution and rapid elimination; the mean (± standard deviation) distribution and elimination half-lives ($t_{1/2}$) were 0.13 (± 0.024) and 1.21 (± 0.21) h, respectively. At steady state, the volume of distribution was 2.36 (± 0.23) l/kg. Elimination of the steroid was rapid with a total clearance of 2.75 (± 0.42) l/h·kg[22].

In adult female rats in proestrus, ^{3}H-progesterone was administered by gastric intubation, subcutaneous injection, or uterine intraluminal instillation. Absorption by the endometrium was rapid, and progesterone was eliminated from uterine tissue in a biphasic pattern with half-lives of 6.5 and 230 min for the α and β phases[23].

Following intravenous injection of ^{3}H-progesterone to non-pregnant monkeys, the labeled hormone disappeared from the circulation by 3 h after administration; between 0.5 and 1.75 h after ^{3}H-progesterone disappeared, it reappeared in the circulation, reaching a maximum mean value of 20% of the initial C_{max}. Progesterone metabolites also reappeared, suggesting that the parent compound was released from tissue stores since progesterone does not undergo enterohepatic recirculation[24].

After oral administration, progesterone is approximately 96–99% bound to serum proteins, primarily to serum albumin and corticosteroid binding globulin[6,11]. Progesterone is metabolized primarily by the liver, largely to pregnanediols and pregnanolones[6]. Pregnanediols and pregnanolones are conjugated in the liver to glucuronide and sulfate metabolites. Progesterone undergoes renal and biliary elimination. Following injection of labeled progesterone, 50–60% of the excretion of metabolites occurs via the kidney; approximately 10% occurs via the bile and feces. Overall recovery of the labeled material accounts for 70% of an administered dose. Only a small portion of unchanged progesterone is excreted in the bile[6,11].

Toxicology

A vaginal progesterone gel[10] and a vaginal progesterone insert[11] are currently approved for clinical use and a progesterone vaginal ring is approved in a few countries in Central and Latin America[25,26]. As progesterone is a physiological hormone, few preclinical studies have been conducted with these formulations and one preclinical toxicity study has been conducted with a progesterone vaginal ring[16]. Several reports issued by various governmental agencies or published literature have evaluated the carcinogenic and mutagenic potential of progesterone as well as its potential to cause reproductive toxicity[16,27–30].

In a review of the reproductive and developmental toxicity of progesterone developed for the California Environmental Protection Agency, no clear relationship had been demonstrated between exogenous progesterone and developmental or reproductive toxicity in humans[30].

Acute toxicity—In mice, the median lethal dose (LD_{50}) of progesterone, given by intravenous injection, is 100 mg/kg; in rats the LD_{50} is 327 mg/kg when the hormone is given by intraperitoneal injection. In rabbits, the LD_{50} was 26.5 mg/kg of body weight[30,31].

Chronic toxicity —In a study in the rhesus monkey, 25 mature females were assigned to treatment with intravaginal rings delivering progesterone (235 or 1770 μg/day), norethisterone (39 or 340 μg/day), or placebo for 52 weeks[16]. Body weight gain and food and water consumption were not affected by treatment. Vaginal ring insertion was associated with a change in the balance of vaginal microflora in both active and control groups. The progesterone and norethisterone groups experienced a reduction in acid-tolerant bacteria; the balance was restored in all groups except the high-dose norethisterone group by the end of the study; no increase in the incidence of Candida species was observed in any group. A significant increase in fibrinogen in the high-dose norethisterone group versus the control group was the only hematologic change (253.4 ± 248.8 vs. 184.6 ± 184.6 mg%; $p < 0.01$)[16].

Carcinogenicity studies

The International Agency for Research on Cancer (IARC) reviewed evidence on the carcinogenicity of progesterone and designated progesterone as 'possibly carcinogenic to humans' (Group 2B), although evidence for carcinogenicity to humans was considered inadequate[27]. The US National Toxicology Program (NTP) has classified progesterone as an agent 'reasonably anticipated to be a human carcinogen' since 1985; this classification is based primarily on evidence from the IARC reports[28]. Neither the IARC nor NTP has found sufficient evidence to conclude that progesterone exposure is linked to the development of cancer in humans.

Newborn female rats receiving progesterone subcutaneous injections and subsequent treatment with a carcinogen DMBA (7,12-dimethylbenz(a)anthracene) had an increased incidence of mammary adenocarcinoma as compared with control animals[31].

In mice treated with subcutaneous implants releasing 59–900 μg of progesterone per day for 18 months, ovarian granulosa cell tumors were found in 27 of 83 treated mice and in one of 33 controls; the tumors were small (< 0.5 mm in diameter). In a similar study in mice treated with subcutaneous implants releasing 18–900 μg/day of progesterone for 18 months, uterine sarcomas were found in 15 of 142 treated mice and no control mice at the end of the study[32].

As noted in the earliest IARC review[32], when combined with known carcinogens (chiefly polycyclic aromatic hydrocarbons), progesterone influenced the incidence and histological type of mammary, uterine, and vaginal tumors in mice, rats, and rabbits[32]. An increase in tumor incidence was only observed when progesterone was given *after*, but *not before*, the carcinogen[32]. A review of rodent studies noted that, in the rat, progesterone increases the frequency of mammary tumors induced by DMBA or MCA (3,4-benzopyrene, 3-methylcholanthrene) in intact and ovariectomized animals but not in ovariectomized-adrenalectomized animals[33]. Further, chronic administration of progesterone to neonatally androgenized rats at various times after DMBA increased the frequency and growth of mammary carcinoma but moderate-to-high doses of progesterone plus high doses of estrogen inhibited mammary carcinoma in this model[33].

In beagle dogs, long-term (74 weeks) subcutaneous progesterone injections in doses of 0.8–22.5 mg/day caused endometrial hyperplasia; no tumors were found on necropsy after the last dose, but fibroadenomatous nodules were found in two of five dogs receiving the highest dose.

In 52 rabbits given subcutaneous injections of progesterone 10 mg twice-weekly and then exposed to vaginal strings containing MCA, vaginal tumor incidence was similar to that of controls after 20 months (4/30 vs. 5/23)[32].

Progesterone or medroxyprogesterone acetate (MPA) appears to have different potentials for carcinogenicity. In virgin female BALB/c mice, the incidence of mammary tumors was lower in those treated with progesterone in silastic subcutaneous pellets than in those treated with MPA, given by the same route at the same dose. In the progesterone-treated group, 67% of tumors were lobular and 33% were ductal; the proportions were reversed in the MPA-

treated group. The ductal tumors were estrogen receptor-positive and PR-positive, while the lobular tumors were not, suggesting different mechanisms for carcinogenesis for the two progestins[34].

Several studies in nulliparous, intact female rats have demonstrated that a combination of an estrogen and progesterone may be protective against the induction of mammary cancer by N-methyl-N-nitrosourea[35,36]. This effect mimics the protective effect of early pregnancy on subsequent development of mammary tumors.

Genotoxicity

In the *Salmonella* mutagenicity assay (Ames test), progesterone was not mutagenic[37]. In the mouse lymphoma assay, evidence for mutagenic activity was difficult to interpret[38]. Progesterone did not induce dominant lethal mutations in mice or chromosomal aberrations in rats treated *in vivo* and did not cause chromosomal aberrations in human or rodent cells or sister chromatid exchanges in cultured human cells. Studies on transformation of rodent cells *in vitro* were inconclusive: a clearly positive result was obtained for rat embryo, but results were weakly positive and negative in mouse and Syrian hamster cells, respectively[27].

Reproductive and developmental toxicity

The offspring of pregnant Wistar rats injected with progesterone (1.5 mg/kg), between gestation days 8 and 21, weighed less at weaning and showed reduced performance on one measure of open field exploration and a slight increase in brain DNA as compared with controls; the effects on brain development were not considered to be drug-related[39].

In pregnant Wistar rats given progesterone 0.1, 1, 5, or 20 mg/kg by intramuscular injection at various times during gestation, the two lowest doses were not associated with effects on fetal or placenta weights, although the frequency of early resorptions increased in rats given 1 mg/kg on gestation days 1–6 and 2.5% of the surviving fetuses had a morphological defect[40]. The highest dose, 20 mg/kg dose, given on gestation days 1–6, led to intrauterine death of all fetuses[40], while the 5 mg/kg dose led to reduced birth weights and placental weights and to an increase in fetal defects (3.0%) in surviving fetuses[40].

Effect of prenatal exposure on sexual development

The effects of prenatal progesterone on *female sexual development* have been studied in rats[40], rabbits[41], mice[42], rhesus monkeys[43,44] and guinea pigs[45]. In mice, the female offspring of pregnant mice treated with 0.25 mg or 0.5 mg subcutaneous progesterone, given on gestation days 12–16 had increased anogenital distances compared with control[42]. When the progesterone-treated female offspring were mated, fertility and pregnancy outcome were unaffected but an increase in aggressive behavior toward male mice was observed during lactation[42].

In a study in rhesus and cynomolgus monkeys, intramuscular progesterone plus estradiol benzoate were given from gestation days 20 to 50 at 0.1–25 times the human dose equivalent

(HDE). Embryo lethality was observed at doses 10 and 25 times the HDE; one dead female fetus (progesterone dose: 10 times HDE) exhibited masculinized external genitalia[43].

The effects of prenatal exposure to exogenous progesterone on *male sexual development* have not been studied extensively in animal models. A change in anogenital distance in male, as well as female, offspring of rabbits exposed to prenatal progesterone was reported[41] but no 'feminization' was reported in three male monkey infants exposed to progesterone[44].

In one study, pregnant female mice were given progesterone 2 mg/day by subcutaneous injection on gestational days 14–16. Anogenital distance and body weight were unaffected by progesterone exposure. At 90 days, however, male mice exposed to prenatal progesterone showed deficiencies in mating behavior, marked by failure to achieve mounting or intromission[46].

Progesterone as a neurosteroid and preclinical studies in the brain

More recently, most preclinical work was geared at identifying the unique role of progesterone on neurogenesis and brain effects.

Progesterone receptors have been identified in several areas of the brain[47] and have been implicated in a variety of functions, including cognition[48], neuroprotection[49,50], and dendritic remodeling[51]. Progesterone reduces the incidence of epileptic activity both directly[52] and through conversion to allopregnanolone[53]. In addition, progesterone has neuroprotective effects after ischemic or traumatic brain injury, both alone and after estrogen priming[54].

Despite positive preclinical studies, failure of clinical trials of traumatic brain injury were discouraging. However, extrapolation from pre-clinical data to humans needs careful dose-finding, and suboptimal dosing was suggested as a factor of failure[50].

In *preclinical studies*, progesterone and some structurally related progestins have shown neuroprotective properties mediated via the PR[55–58].

Preclinical studies showed that progesterone and Nestorone, with or without estradiol, stimulate oligodendrocytes and myelin repair in both *in vitro*[55] and *in vivo*[56] experiments and decrease the severity of the disease induced in experimental autoimmune encephalomyelitis[57], an animal model for multiple sclerosis, with consistent responses in the same dose range. Progesterone and Nestorone were shown to promote myelin repair by binding to PRs, thereby stimulating recruitment and maturation of oligodendrocyte progenitor cells. In contrast, other commonly used synthetic progestins such as levonorgestrel and medroxyprogesterone acetate have potential adverse outcomes on the brain's regenerative capacity[55,58].

Brinton's laboratory[58] reported the efficacy of different progestins used alone or in combination with 17β-estradiol on adult rat neural progenitor cell (rNPC) proliferation and hippocampal cell viability. *In vitro* analyses indicated that progesterone, norgestimate, Nestorone, norethynodrel, norethisterone, and levonorgestrel significantly increased rNPC

proliferation, whereas norethisterone acetate was without effect, and MPA inhibited rNPC proliferation. Proliferative progestins *in vitro* were also neuroprotective. Acute *in vivo* exposure to progesterone and Nestorone significantly increased proliferating cells.

Experimental studies also showed progesterone efficiency in reducing infarct volume and improving functional recovery in a model of transient middle cerebral artery occlusion[59–61].

Allopregnanolone may also protect the brain against ischemic damage by other signaling mechanisms not involving PRs[62]. Liu and colleagues[63] have shown that Nestorone was also effective in that model. PRs are mediators of neuroprotection, as progesterone is not effective in PRKO mice[63]. Also, progesterone and Nestorone were less efficient in heterozygous mice, expressing reduced levels of PRs[56,63].

In addition, Brinton's laboratory showed the efficacy of allopregnanolone as a regenerative agent for Alzheimer's disease and with potential to treat multiple neurological disorders[64–66].

Conclusion

Progesterone is a physiological hormone to which women are exposed at high levels during pregnancy. The various formulations developed for therapy, either oral or from other delivery systems, had few preclinical studies conducted but the clinical data support the benefits of this therapy in the approved indications.

It is possible that progesterone and related molecules may also confer novel therapeutic benefits in the nervous system for the treatment of stroke, brain injury, myelin repair and Alzheimer's disease.

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