ANTIFERTILITY EFFECTS OF 2 α,17 α-DIETHYNYL, A-NORANDROSTANE, 2 β,17 β-DIHYDROXY, 2 β-SEMISUCCINATE (AF-57)

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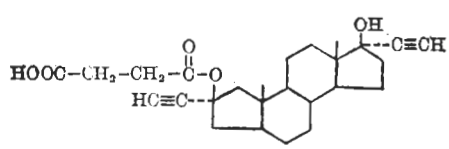
ABSTRACT AF-57, a analogue of anordrin, demonstrated antifertility effects in rats, mice, hamsters and rabbits, depressed the function of corpora lutea in rabbits and caused a retardation of cleavage and degeneration of ova in hamsters. The estrogenicity of AF-57 was lower than that of anordrin in mice, but similar to anordrin in rats. AF-57 showed a higher binding ability than anordrin to cytosol estrogenic receptors of uteri in rats.

KEY WORDS 2 α, 17 α-diethynyl, A-norandrostane, 2 β, 17 β-dihydroxy, 2 β-semisuccinate (AF-57); oral contraceptives; ovum transport; estrogen receptors.

Anordrin (2 α, 17 α-diethynyl, A-norandrostane, 2 β, 17 β-dihydroxy dipropionate) is an anti-implantation agent with estrogenic activity in rats, mice, rabbits and hamsters. It has been used as a "vacation tablet" given to women on one month's vacation in China[1,2]. The contraceptive efficacy was 99.5% for 6056 woman-months or 94%for 505 woman-years[3]. The derivatives of anordrin have been synthesized and screened for compounds having higher anti-fertility effect but lower estrogenicity potency. AF-57, an analogue of anordrin with the 2 β, 17 β-dipropionate replaced by 2 β-succinate and 17 β-hydroxy group, was tested for antifertility effect. The chemical name is 2 α,17 α-diethynyl, A-nor-androstane, 2 β, 17 β-dihydroxy, 2 β-semisuccinate.



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MATERIALS AND METHODS

AF-57 and anordrin were synthesized by Dr LI Rui-lin* at Shanghai Academy of Pharmaceutical Industry. Both compounds were dissolved in salad oil.

Antifertility tests in rats and mice Adult ♀ rats weighing 218±SD 22 g or mice weighing 34±6 g were maintained at animal quarters under lighting from 7 AM to 7 PM. Two ♀ were caged with one proven ♂ and the day appearance of vaginal plugs or sperms was designated as d 1 of gestation. Test compounds were given ig in the morning for various days after mating. Animals were killed on the 6 th day after withdrawal of medication. The number of pregnant animals, implantation sites and fetuses were recorded.

Termination of early pregnancy in rabbits ♀ rabbits weighing 3.7±0.6 kg were mated twice with ♂. Test compounds were ig on d 7–9 after mating. Autopsy was performed on the 6 th day after withdrawal of medication and the number of fetuses were counted.

Anti-implantation test in hamsters Adult hamsters 116±25 g in weight were maintained at 20–23℃ under lighting from 7 AM to 9 PM. Each ♀ was caged with one ♂ hamster and a vaginal smear was taken every morning. The day of finding sperms was designated as d 1. Test compounds were given ig on d 1–2 or 1–3. The hamsters were killed on d 6 and the number of implantation sites were counted.

Test of estrogenic activity Immature mice or rats of the age of 21 d were divided

into 10 groups, with 8–10 animals in each group. Two doses of test compounds and 3 doses of ethynylestradiol in oil were injected sc for 3 d. The control group was given the oil only. Uteri were excised 24 h after the last medication. The ratio of uterine weight (mg)/body weight (g) was taken as the potency of estrogenic activity.

Ova transport and development Mated hamsters weighing 106±18 g were fed AF-57 or anordrin at 9 AM on d 1–3 at a daily dose of 10 mg/kg. Hamsters were killed at 3 PM on d 3 or at 6:30 AM on d 4 for collecting of ova. The hamsters killed at 6:30 Am on d 4 were injected iv with 0.2 ml 1% Niagra sky blue in 0.9% NaCl 15 min before sacrifice to identify the implantation sites. The number of corpora lutea were counted. The uteri and oviducts were flushed with Tyrode's solution and the flushings were examined under a dissecting microscope for ova. The recovered ova were stained with 0.2% Lacmoid in 45% acetic acid and examined under a phase-contrast microscope. Ova with irregular or missing nuclei were considered as degenerated.

Serum progesterone level in pregnancy rabbits Blood was collected on various days after mating from the ear vein of rabbits weighing 3.7±0.6 kg and treated with AF-57 or anordrin (oil only for control group). The blood was kept at 20℃ for 4–6 h and then centrifuged at 3000 rpm for 20 min. The serum was stored at −20℃ until radioimmunoassay for progesterone.

Binding ability to uterus cytosol estradiol receptor in mature rats The uteri were homogenized in iced TES buffer(10 m*M* Tris-HCl, 1.5 m*M* EDTA, 2 m*M* mercaptolethanol, pH 7.4). Cytosol (supernatant) was prepared by centrifuging the homogenates at 20,000×*g* for 1 h at 4℃. Protein was measured with bovine serum albumin as the standard[4]. Cytosol of 200 μl was incubated for 18 h at 4℃ with 1 n*M* [^{3}H] estradiol (107 Ci/m*M*) purchased from New England Nuclear Corp, Boston MA USA in the presence/absence of a 1,000 fold excess of stilbestrol. To measure the ability of competitive inhibition, AF-57 or anordrin was added to the reaction tubes of total binding. DCC of 200 μl (0.5% charcoal and 0.05% dextran in TES buffer) were added to the incubated tubes and then centrifuged for 15 min at 1,000×*g* to separate the bound and free [^{3}H]estradiol. Results were expressed by the % inhibition of the [^{3}H]estradiol binding to cytosol receptor and the competitive inhibition curve were calculated by regression equation.

RESULTS

Antifertility efficacy in rats, mice, rabbits and hamsters AF-57 showed the effects of anti-implantation and terminating early pregnancy in animals that were treated after mating (Tab 1).

Comparision on antifertility and estrogenicity of anordrin and AF-57 The antifertility ED_{50} of AF-57 calculated by the rate of fertility (Tab 2) in mice were slightly lower than that of anordrin ($p>0.05$). Four point assay showed that the estrogenicity of AF-57 was lower than that of anordrin in mice, but similar to anordrin in rats ($p>0.05$).

Ova transport and development in hamsters At 6:30 AM of d 4, 90% of the ova recovered from the uteri of control hamsters were at the blastocyst stage (Tab 3). However, no ovum developed into blastocyst in hamsters treated with AF-57 or anordrin. Instead, only a few ova at 4–8-cell stage were found. When examined at 3 PM of d 3, all ova recovered from the uteri of control hamsters were at the 8-cell stage, while ova recovered from uteri in the treated hamsters were at 4-cell stage or under degeneration. Obviously, treatment with either AF-57 or anordrin after mating caused a retardation of cleavage and degeneration of ova.

Serum progesterone level in pregnant rabbits Comparing the serum progesterone in treated pregnant rabbits with the control, did not show any increasing profile, but started to decline after gavage of 2.5 mg/kg daily on d

Tab 1. Antifertility effect of AF-57. *p>0.05, **p<0.05, ***p<0.01

	Daily dose (mg/kg)	Medication on	Animals Dosed	Animals Pregnant	Fetuses Alive	Fetuses Dead
Mice	0	—	10	10	93	0
	1.36	d 2	10	2***	9***	0
	5.0	d 8	8	2**	11***	34
Rats	0	—	10	8	79	0
	1.0	d 1	10	0****	0***	0
	1.0	d 2	10	0***	0***	0
	1.0	d 3	10	3**	10***	2
	1.0	d 7-9	10	1***	4***	5
	2.0	d 7-9	10	0***	0***	0
Hamsters	0	—	13	7	93	0
	10	d 1,2	8	4*	10***	0
	10	d 1-3	9	1***	0***	6
	10	d 1-4	5	0***	0***	0
Rabbits	0	—	6	6	45	1
	2.5	d 8-10	5	2*	7***	19

Tab 2. Anti-implantation ED_{50} and relative uterotrophic potency of anordrin and AF-57 (95% fiducial limits)

		AF-57	Anordrin
ED_{50}mg/kg	mice	0.7(0.4-1.0)	1.2(0.7-2.0)
Uterotrophic potency	mice	27(2-53)	100
	rats	101(72-134)	100

8-10 (Fig 1). This result suggested that the function of corpora lutea in pregnant rabbits was depressed.

Binding ability to uterine cytosol estradiol receptors in adult rats The K_a of [^{3}H]estradiol binding to cytosol receptor was 1.84 nM^{-1} measured by Scatchard plot. The 50% inhibition doses of AF-57 and anordrin were respectively 0.5 ± 0.3 and 3.7 ± 0.1 μM.

Tab 3. Effects of anordrin and AF-57 on ova development. ***p<0.01

Time of autopsy	Drug	Hamsters	Corpora lutea	Ova in uteri Normal	Ova in uteri Degenerated	Ova in oviducts Normal	Ova in oviducts Degenerated
3:00 PM	—	6	93	80	0	8	0
on d 3	Anordrin	5	71	26***	21	0	0
	AF-57	6	72	45***	14	6	0
6:30 AM	—	6	60	58	0	0	0
on d 4	Anordrin	6	61	7***	14	0	0
	AF-57	5	45	2***	8	2	3

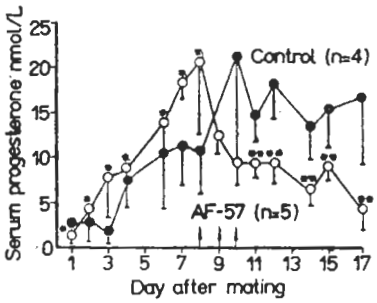

Fig 1. Serum progesterone level in pregnant rabbits. Medications on d 8-10 (2.5 mg/kg daily) by gavage. $\bar{x}\pm SD$

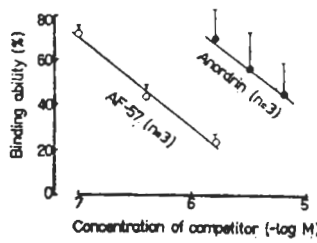

Fig 2. Competition of [^{3}H]estradiol binding to uterine cytosol estrogen receptors in rats. $\bar{x}\pm SD$

Apparently anordrin and AF-57 had specific affinity to cytosol estradiol receptors, while the binding ability of AF-57 was higher than that of anordrin. (Fig 2)

DISCUSSION

AF-57, like anordrin, had antifertility effects with estrogenicity in species, depressed the function of corpora lutea as evidenced by a decrease of serum progestin level and disturbed the transport and development of ovum just as anordrin did[5,6]. Measuring the ED_{50} of anti-implantation effect and estrogenic potency of AF-57 showed a slightly higher antifertility effect but a lower estrogenic potency than that of anordrin in mice. These results suggest that the estrogenicity may not be a unique contributor of the antifertility effect of AF-57. In addition, the binding ability of AF-57 to uterus cytosol estradiol receptor of rats *in vitro* is higher than that of anordrin which was expressed by the dose of 50% inhibition of the [^{3}H]estradiol binding to cytosol receptor. A contradiction is raised from the above mentioned data that AF-57 induced a relatively lower estrogenic activity than that of anordrin *in vivo*, though AF-57 exhibited a higher estradiol receptor binding *in vitro*. It is suggested that the existence of the free hydroxy group in 17-position confers the higher binding ability of AF-57 to cytosol estradiol receptor *in vitro* by referring to the ideas of others[7,8].

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2α,17α-双乙炔,A-失碳雄甾烷,2β,17β-双羟基,2β-单琥珀酸酯(AF-57)的抗生育作用

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提要 AF-57是双炔失碳酯的同类物, 对大鼠、小鼠、仓鼠和兔有明显的抗着床和抗早孕作用, 使仓鼠受精卵变性, 家兔早孕黄体功能受抑制. AF-57在小鼠或大鼠的雌激素活性比双炔失碳酯低或相仿, 但在体外AF-57与大鼠胞浆雌激素受体的结合力明显强于双炔失碳酯.

关键词 2α, 17α-双乙炔,A-失碳雄甾烷, 2β,17β-双羟基, 2β-单珀琥酸酯(AF-57); 口服避孕药; 卵子输送; 雌激素受体