Reproductive Mechanisms Influenced by a Diazocine. (30637)

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Of interest to basic problems of reproductive biology and to applied problems of population control are pharmacologic agents which interfere with selective reproduction processes and yet are reversible in their effects and relatively free of systemic toxicity. This paper describes the effect of 2,8-dichloro-6,12-diphenyl-dibenzo[b,f][1,5] diazocine (U-10293, Fig. 1) on female reproductive mechanisms. This diazocine analog is chemically unique among those compounds reported to interrupt pregnancy in mammals (1).

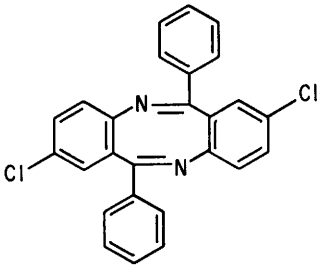

FIG. 1. U-10293 (2,8-dichloro-6,12-diphenyl-dibenzo[b,f][1,5]diazocine).

Methods and results. All rats were fed a recommended Purina laboratory diet and were maintained in an environment of 75 ± 2°F and 14 hours of artificial light daily. Non-steroidal compounds were administered in water containing .25% methylcellulose; steroids were administered in sesame oil containing 5% benzylalcohol.

Pregnancy inhibition activity was assessed in mature (200 g) nulliparous rats. U-10293 treatment was initiated during proestrus (Day 0) and was continued daily for a total of 7 doses. All females were mated with males of known fertility within 24 hours of the first treatment and autopsied 48 hours after (Day 8) last dose. Assessment of pregnancy inhibition was made by gross examination of uteri for implantation sites. Minimal doses of U-10293 causing complete inhibition of pregnancy were 0.5 mg orally and 1.0 mg subcutaneously (3 to 5 rats/dose). Below these effective doses, numbers of implantation sites per rat were equivalent to colony average of 10.7.

Effect of shorter intervals of treatment was evaluated at autopsy on Day 20. By gavage, a 10 mg daily dose completely inhibited pregnancy whether administered from Day 0 through Day 3, Day 3 through Day 6, or on Day 2 or Day 4 (Table I). A single dose on either Day 10 or Day 15 had no significant inhibitory effect on the number of developing fetuses whereas a single dose on Day 6 resulted in a diminution of number of pups/litter and a concomitant increase in the number of resorption sites. Subcutaneously, U-10293 inhibited pregnancy when administered either from Day 0 through Day 3, Day 3 through Day 6 or on Day 2. Treatment on either Day 4, 6, 10 or 15 of pregnancy did not affect average pup weight, number of pups per litter or number of resorbing sites per litter.

Effect on fetal development was checked using pregnant rats subjected to 1.0 mg daily, orally, of U-10293 from Day 8 through Day 15 of pregnancy (one group of 7 rats) and from Day 15 through Day 20 of pregnancy (a second group of 8 rats). Uterine contents of all rats were examined at sacrifice on Day 21. Of 7 animals treated during mid-pregnancy, 2 showed no indication of implantation sites at time of autopsy. The remaining 5 animals contained a total of 9 living pups and 15 early resorbing implantation sites. Live fetuses appeared grossly normal. No effect of U-10293 on fetal development occurred with treatment during advanced pregnancy. These 8 pregnant rats averaged 10 live fetuses per litter, with a range from 8-12. No gross anatomical defects of fetuses or abnormal numbers of resorbing implantation sites were observed. The acute LD_{50} in mice of U-10293, administered intraperitoneally, was >1,000 mg/kg.

Uterotropic activity was assessed in bilaterally ovariectomized, immature (100 g) rats following 10 days of consecutive treatment.

TABLE I. Pregnancy Inhibiting Activity of U-10293 in Rats Following a Restricted Number of Treatments (Daily Dose of 10 mg).

Treatment period (days of pregnancy)		No. preg/No. trt		Avg pup wt (g)		Avg No. pups/ litter		Avg No. resorption sites	
U-10293	0–3	0/5*		—		—		—	
			0/4†		—		—		—
	3–6	0/5		—		—		—	
			0/5		—		—		—
	2	0/5		—		—		—	
			1/5		—		—		—
	4	0/5		—		—		—	
			4/4		2.3		9.7		1.2
	6	4/4		2.6		4.2		5.6	
			5/5		2.3		9.7		1.0
	10	5/5		2.4		9.8		.6	
			4/4		2.0		10.2		.0
	15	5/5		2.1		11.4		.6	
			5/5		2.3		11.2		1.8
Vehicle No. 122	3–6	5/5		2.4		9.0		.0	
			5/5		2.4		10.0		1.8

* Data for oral administration. † Data for subcutaneous administration.

U-10293 enhanced uterine weights at oral doses of 10, 25, 50 and 100 μg daily (Table II). Its potency, relative to orally administered diethylstilbestrol, was 10% (95% confidence limits = 5% to 19%).

Gonadotropin inhibiting properties were evaluated using intact, immature (100 g) male rats subjected to 10 days of consecutive treatment. At daily oral doses of 80 μg and above, U-10293 effectively suppressed testicular and accessory sex organ weights (Table III). Magnitude of depression of organ weights was comparable to that achieved with diethylstilbestrol—potency of U-10293 in this assay was 1.8% (95% confidence limits = 0.7 to 2.9%) of diethylstilbestrol.

Changes in vaginal epithelial cytology were observed in mature bilaterally ovariectomized rats treated for 8 consecutive days at a daily oral dose of 1 mg of U-10293. Cornified epithelial cells predominated in vaginal smears of each of 6 treated rats within 3 days of starting treatment. Typical estrous smears persisted for 2 to 14 days after end of treatment.

TABLE II. Uterotropic Activity of U-10293 in Immature Female Rats (Five rats/point).

Compound	Daily oral dose (μg)	Uterine wt & range (mg)		Body wt increase (g)
U-10293	.0	35	(31-45)	45
	10	43	(32-69)	31
	25	92	(52-144)	21
	50	118	(78-140)	32
	100	125	(88-154)	25
Diethyl-stilbestrol	.5	30	(23-41)	42
	1.0	66	(50-78)	31
	10	123	(101-133)	31

Vaginal cytology was also followed in mature intact rats having established normal estrous cycles (5 rats/group). Daily treatment with 10 μg orally of U-10293 was initiated during proestrus and continued for a total of 10 days. Vaginal smears were obtained throughout treatment and after treatment until a normal estrous cycle was observed. Cornified epithelial cells predominated smears during treatment. Just prior to or immediately following the end of treatment, leucocytic smears occurred and persisted for 4-6 days before typical estrous cycles reoccurred. Administered subcutaneously, a similar effect during treatment was observed; however, recovery of normal cyclic vaginal changes did not resume until 10-36 days after end of treatment. One mg of estradiol daily, subcutaneously, maintains rats in an anestrous condition for 9-10 days after end of treatment.

Effects of blastocyst implantation were evaluated using mature rats injected with

TABLE III. Gonadotropin Inhibiting Activity of U-10293 and Diethylstilbestrol in the Immature Male Rat (Oral Administration, 5 rats/point).

Compound	Daily dose (μg)	Testes wt (mg)	Seminal ves wt (mg)	Prostate wt (mg)	Body wt (mg)
U-10293	.0	1204 ± 64.1	16 ± 1.1	100 ± 4.97	51
	40	1209 ± 119.6	16 ± 2.19	112 ± 9.6	46
	80	835 ± 49.8*	11 ± .94†	65 ± 6.49*	40
	160	708 ± 115.8*	11 ± .49†	69 ± 8.05*	34*
	500	512 ± 7.8*	14 ± 1.12	57 ± 4.61*	25*
	1000	544 ± 54.9*	15 ± 1.57	50 ± 5.10*	20*
Stilbestrol	2.5	728 ± 60.7*	12 ± .51†	82 ± 4.26	45
	5	429 ± 45.8*	17 ± 1.80	61 ± 2.27*	46
	10	450 ± 10.3*	18 ± 1.12	60 ± 2.84*	33*
	20	516 ± 24.2*	16 ± .55	65 ± 2.08*	32*
	40	377 ± 50.9*	19 ± 1.24	55 ± 2.11*	28*

* Significantly ($P < .05$) different from controls; † Borderline significance at $P = .05$.

TABLE IV. Implantation in Provera-Treated Rats Following Administration of U-10293 (Oral) or Estrone (SQ) from Day 1 Through Day 12 of Pregnancy.

Compound*	Daily dose (μg)	No. rats treated	Uterine contents†	
			Blastocysts	Implantation sites
—	—	4	3-7-8-12	—
U-10293	1	5	0-6-8-9-10	—
	10	5	0	3-6-8-10
	100	5	0	2-7-12-13
Estrone	1	5	0	4-8-10-10-11
	10	5	0-0-0-0-0	—
	100	5	0-0-0-0-0	— Uteri all very enlarged.

* All rats also received Provera 1.5 mg, SQ) from Day 1 through Day 12.
† No. per rat at sacrifice on Day 12.

Provera* (1.5 mg daily initiated on Day 1) to induce delayed implantation. This regime was supplemented with either estrone (1, 10 or 100 μg/day, subcutaneously) or U-10293 (1, 10 or 100 μg/day, orally) from Day 1 through Day 12. Rats were autopsied on Day 12 and their uteri examined for evidence of implantation. Uterine cornua were flushed with 0.9% saline in an attempt to recover blastocysts if implantation had not occurred. Ten or 100 μg of U-10293 and I μg of estrone induced implantation (Table IV). At higher doses of estrone no blastocysts or implantation sites were observed. In absence of either U-10293 or estrone, unimplanted blastocysts were recovered on Day 12.

Discussion. U-10293 effectively inhibited pregnancy following either oral or subcutaneous administration and was relatively non-toxic. As such, it is a significant addition to the small number of compounds available which achieve antifertility efficacy without associated systemic toxicity to pregnant females(1). It appears, however, that the pregnancy inhibiting effects were associated with estrogenic properties. Uterine weight increases in immature rats are one index of estrogenic activity. U-10293 induced a response typical of that observed with frank estrogens. Additional estrogenic responses observed were cornification of vaginal epithelial cells in both ovariectomized and intact immature rats and reduced testicular and ventral prostate weights of intact immature male rats. Increasing seminal vesicle gland weights (at 500 and 1000 μg doses of U-10293) are also characteristic of responses invoked by administering testicular weight suppressing doses of estrogens.

Following termination of subcutaneous treatment, normal cyclic vaginal smears did

* Upjohn Co. registered trademark for medroxyprogesterone acetate.

not resume until 10-36 days later. Under similar test conditions a 1 mg daily dose of Provera, a compound with pronounced depot activity in domestic animals and man, induces an anestrous condition which persists for approximately 20 days following termination of treatment (unpublished data). These results suggest U-10293 may have significant depot activity in other test animals.

Induction of implantation with U-10293 was likewise consistent with an estrogenic effect. Blastocysts in Provera-treated intact rats remain free in the uterine cavity until a critical amount of exogenous estrogen is provided(2). Increasing estrogen doses beyond that required for nidation results in a loss of blastocysts from the uterine cavity. For example, Nutting and Meyer(3) reported 0.3 μg of estrone is a minimum dose supporting implantation in progesterone-treated castrate rats. Daily doses of 1 and 3 μg, but not 10 μg, were compatible with implantation. Their findings were comparable to results reported here with estrone in Provera-treated intact rats and are what would be anticipated with higher doses of U-10293.

Summary. 2,8-Dichloro-6,12-diphenyl-dibenzo[b,f][1,5] diazocine completely inhibited pregnancy when administered either orally or subcutaneously to female rats at doses as low as 0.5 mg daily for 7 days at time of mating. At higher doses, single treatments prior to completion of implantation were equally effective. Systemic or fetal toxicity did not occur at therapeutic doses. This compound also induced uterine growth, vaginal cornification, blastocyst implantation and inhibited gonadotropin activity. Each of these latter effects are indicative of estrogenic properties, and are probably involved with antifertility efficacy. This non-steroidal compound is a structurally unique addition to those agents with these biological activities.

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Hemolysin Formation in Newborn Mice of Different Strains.* (30638)

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The immune response of adult mice to sheep red blood cells (sRBC), measured by the Jerne technique(1), is known to be different in different strains(2). Thus Friedman found that adult AKR mice are the poorest responders among several strains tested(2). In contrast, we observed that newborn AKR mice give good early responses to sRBC as judged by numbers of hemolysin-forming cells (3). We therefore decided to extend our tests to 2 additional strains. This communication presents the results of these comparative studies during which we also tested strain differences in regard to the previously noted ability of oligodeoxyribonucleotides to stimulate immune responses(3,4).

Materials and methods. Pregnant female mice, one week before term, were obtained from the following sources: Ha/ICR from Millerton Research Farms, Millerton, N. Y.; C_{57}Bl/6 and AKR from Jackson Laboratory; Bar Harbor, Maine. The newborn animals of these mothers were immunized intraperitoneally between the third and tenth day of age, depending on the experiment, with 10^8

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