Antispermatogenic Properties of 2,3-Dihydro-2-(1-naphthyl)-4(1H)-quinazolinone (U-29,409) (35614)

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Compounds which suppress spermatogenesis include many chemical classes and modes of action (1). None of the known compounds meet requirements necessary for a marketed male contraceptive. Reasons which exclude human use of present antispermatogenic compounds fall categorically into two broad areas of general toxicity and of potential mutagens. A further disclaimer for such compounds is their general action on specific stages of spermatogenesis (2, 3); azoospermia occurs weeks or months later when the more mature germinal cells have developed and been depleted from the excurrent ducts.

Complete inhibition of sperm production offers a reliable method of antifertility. Practical application calls for oral or injectable administration with a rapid onset of infertility. The contraceptive effect must not be associated with adverse properties like lowered libido or a decline in general health. One method of achieving the above criteria is to chemically cause immature germinal cells to exfoliate. This action would produce mass migration of cells through the male reproductive tract and shorten the time to infertility. A highly selective nonendocrine condition exists as a vulnerable point for such action—the intercellular bridges which connect some immature germinal cells (4, 5). The compound under study (U-29,409) causes cells of the seminiferous epithelium to exfoliate and migrate from the testis to excurrent ducts with an ensuing early and prolonged infertility. An unknown chemical event displaces these cells, however, at present, consideration is given to U-29,409 cleaving the intercellular bridges.

Materials and Methods. Spartan and Upjohn male (300 to 450 g) rats were used throughout under controlled laboratory conditions. U-29,409 [2,3-dihydro-2-(1-naphthyl)-4(1H)-quinazolinone] was administered in 0.25% methylcellulose vehicle by sc and oral routes, in a male antifertility assay (6) to determine efficacy. This assay requires 8 days of daily treatment followed by a check on fertility (mating of males to nontreated females). Two days after withdrawal of treatment the males are autopsied and observed for gross abnormalities of testes and epididymides. These same tissues are then fixed in Bouin's solution and stained with hematoxylin and eosin for histological observation.

U-29,409 was also tested at various doses and routes of administration for 8 and 21 days and the males were placed on a weekly mating schedule (7). Weekly cohabitation with normal cycling females permitted a check on the onset of infertility and return to fertility. Another study was designed for 14 days of oral drug treatment (15 mg/rat) with a treated and control rat killed every week for 8 weeks in order to obtain data on testicular and epididymal histology. Two other studies were performed to learn if U-29,409 is effective after one dose—either by oral intubation or fed in a bait. The bait was Top Choice (General Foods Corporation) dog food (5.2 g mixed with 0.8 ml of H_2O) made in cubes of 6.0 g which contained 50 mg or 100 mg of U-29,409. Effectiveness was measured 7 days later by gross and microscopic examination of testes and epididymides.

U-29,409 was later taken up in vehicle which contained 5 mg of carboxymethylcellulose, 4 mg of polysorbate 80, 9 mg of sodium chloride, and 9 mg of benzyl alcohol/ml. Male rats were given a single ip dose of 50 or 75 mg/kg and killed 30 or 48 hr later. The initial segment of each caput epididymidis

TABLE I. Fertility of Male Rats Treated by Different Routes, Amounts, and Length of Time with U-29,409.[a]

Group (mg/rat) days	Rat no.	No. of implantation sites; weeks: 1	2	3	4	5	6	7	8	9	10	11	12	13
5, sc 21	1	11	15	0	0	0	13	8	12	0				
	2	16	0	12	0	2	13	13	X	9				
	3	X	15	14	0	X	X	6	13	14				
15, sc 8	1	8	9	13	X	13	13	X	15	10				
	2	0	5	0	0	5	9	12	11	14				
	3	9	4	10	X	12	X	4	12	13				
15, oral 8	1	4	0	0	0	0	0	0	X	0	X	0	0	X
	2	1	0	1	X	0	0	0	0	0	0	5	10	8
15, oral 14	1	12	X	A										
	2	14	9	A										
	3	13	X	1	A									
	4	13	0	0	0	A								
	5	5	0	0	0	0	A							
	6	16	13	2	0	0	0	A						
	7	9	0	0	X	0	0	0	A					
	8	15	1	1	0	0	0	X	0	A				
Control, oral 14	1	10	11	A										
	2	14	13	11	A									
	3	14	11	14	6	A								
	4	15	11	0	13	6	A							
	5	12	X	10	13	8	12	A						
	6	15	X	X	13	8	0	12	A					
	7	0	X	14	13	12	X	8	17	A				
	8	8	0	14	X	X	15	0	15	A				

[a] X = did not mate; A = autopsied.

was incised and pressed gently to release its contents into culture medium TC-199. Sperm were checked for motility and all cells were counted in a hemacytometer.

Results and Discussion. U-29,409, when given orally for 8 days at 15 mg/rat, causes mass migration of germinal elements out of the testes and into the efferent ducts and epididymides. Marked distention of the cell filled efferent ducts is visible to the unaided eye. Epididymides, sometimes swollen, contain a mixture of immature germinal cells and sperm; whereas the caput is filled mostly with immature germinal cells, the cauda has mostly sperm—with a high incidence of heads separated from tails. Spermatoceles and sperm granulomas develop in a few rats, at all effective doses. These anomalies are located primarily in the ductuli efferentes and initial segment of the caput epididymidis. Mass invasion of seminiferous epithelium into the excurrent ducts causes distention and stress which could develop into an occlusion resulting in the aforementioned anomalies. Rats may be particularly vulnerable to epididymal stress as α-chlorohydrin (U-5897) produces an epididymal lesion in the adult rat but not in other tested species (8).

Table I shows results of experiments to test the antifertility effectiveness of U-29,409 under different conditions. The ability of U-29,409 to bring about infertility in the male rat is unquestioned. The route and amount of compound given regulates the severity of spermatogenic arrest. U-29,409 is more potent orally than when administered sc; it has

TABLE II. Effectiveness of U-29,409 as an Antifertility Compound When Fed Once to Rats in Top Choice Dog Food.

Group	Rat no.	Treatment	Wt of remaining food (g)[a]	Spermatocele (efferent ducts)	Testes	Epididymides
I	1	Two 6.0-g cubes	0	No	Normal	Normal
	2		0	No	Normal	Normal
	3		0	No	Normal	Normal
II	1	Two 6.0-g cubes	5.00	Yes	Flaccid and small	SCO[b]
	2	with 50 mg of	7.43	Yes	Flaccid and small	SCO
	3	U-29,409/cube	9.19	Yes	Swollen	Swollen; sperm granuloma in caput
III	1	Two 6.0-g cubes	6.73	Yes	Flaccid	SCO
	2	with 100 mg of U-29,409/cube	5.10	Yes	One swollen; one flaccid	Swollen and SCO
	3		3.77	Yes	One normal; one flaccid	SCO

[a] Bait given to males overnight in lieu of regular diet.
[b] SCO = Sperm in cauda only.

poor solubility in water and perhaps slower uptake from a sc site. Low sc doses (5 mg/rat for 21 days) suppress later stages of spermatogenesis and prevent fertility during weeks 4 and 5. Higher oral doses (15 mg/rat for 8 days) inhibit spermatogenesis to spermatogonia and stem cell stages. Rats in which spermatogenesis is inhibited or arrested at the spermatogonial stages maintain their infertile condition for at least 10 weeks (9). Libido, as measured by ability and desire to mate, remains unimpaired. This sexual behavior plus histological morphology infers that U-29,409 does not directly effect Leydig cells.

Placing U-29,409 at effective concentrations in bait provides an opportunity to test drug palatability and acceptance. Although rats reduce their feed intake when confronted with treated bait, all consume sufficient amounts to inhibit spermatogenesis (Table II). A single oral dose of U-29,409 in aqueous vehicle becomes effective at around 100 mg/kg of body wt. Rats given U-29,409 (total of 12, at 2/group) in 100 mg/kg increments through 500 mg/kg have smaller testes and epididymides with depleted sperm when seen 8 and 14 days later. These results are verified when checked histologically in that spermatogenesis is suppressed; controls are normal.

An experiment to produce maximum exfoliation of seminiferous epithelium is an end result of the above studies. Suspending U-29,409 in a different vehicle and administering one ip dose are attempts to solve the solubility and perhaps absorption difficulties. Table III provides data which attest to the pronounced effect U-29,409 has on the testes. The 10^7 numbers of immature germinal cells recovered within 48 hr from initial segment of the caput epididymidis give ample evidence of outward migration of these testicular cells. The prematurely released testicular sperm are weakly motile and are present in the cell population of spherical germinal cells at an average ratio of 1:6 (Table III).

Some doubt exists as to the complete (100%) reversibility to the fertile state after

TABLE III. Numbers of Immature Germinal Cells Recovered from Initial Segment of the Caput Epididymidis within 48 hr of U-29,409 Treatment.

Dose[a] (mg)	No. of rats	Time after treatment (hr)	Av no. of cells × 10^6	
			Sperm	Immature germinal cells
50	2	30	5.0	5.5
50	8	48	8.6	13.7
75	5	48	8.0	47.6
Control	5	48	33.8	0.0

[a] Single ip dose (mg/kg of body wt).

treatment with U-29,409. One explanation for a nonreturn of fertility is, as previously mentioned, the occluded excurrent ducts remain permanently blocked and thereby initiate a secondary inhibition of spermatogenesis. Based on histological evidence, spermatogenesis is complete (in most animals) upon recovery from U-29,409 treatment. There are, however, some seminiferous tubules in these same animals which contain only Sertoli cells. The hypothesis is that U-29,409 works, at least in part, by cleaving the intercellular bridges between developing germinal cells. And if the rat, like one primate species (5), has spermatogonial cells which interconnect by intercellular bridges, the loss of such a connection may weaken the anchoring effect to the tubular membrane. Various segments of seminiferous tubules are synchronized to different stages of spermatogenesis and this may account for variability of U-29,409 suppression. Whatever the mechanism, it is known that U-29,409 causes an immediate, profound exfoliation of immature germinal cells and their subsequent exit from the testis.

Summary. U-29,409 [2,3-dihydro-2-(1-naphthyl)-4(1H)-quinazolinone] is an antifertility compound in the rat. Antifertility is due to inhibition of spermatogenesis—which is accomplished by sc, ip, or oral treatment administered as single or multiple doses. Lowered fertility commences in the second week and may continue, depending upon severity of the dose, through the tenth week. Libido, as measured by ability and desire to mate, is not altered. U-29,409 affects spermatogenesis with a massive exfoliation of immature germinal cells which then migrate from the testis to the excurrent ducts.

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