Antiarthritic Effects of the Antispermatogenic Agent 2,3-Dihydro-(1-naphthyl)-4-(1*H*)-quinazolinone (U-29,409) (35871)

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Ericsson (1) has reported that U-29,409 causes cells of the seminiferous epithelium to exfoliate and migrate from the testes to excurrent ducts. This action elicits prolonged infertility in male rats. The biologic mechanism (s) responsible for this action is unknown. But displacement of intercellular bridges between developing germinal cells is postulated (1).

The effect of U-29,409 on experimental arthritis, acute inflammatory reactions and allergic encephalomyelitis is discussed below.

Methods. The assessment of drugs in acute carrageenan-induced edema, yeast-induced fever, and chronic adjuvant-induced polyarthritis of rats has been described on several occasions (2–6).

Allergic encephalomyelitis is produced routinely by the intradermal inoculation of 0.1 ml of 6–10% lyophilized guinea pig brain (Pentex) in Freund's complete adjuvant (FCA) into the tail and both hindpaws, or 0.3 ml into the tail alone, of Carworth female rats, weighing 150–175 g. Rats develop symptoms of paralysis (ataxia, urinary incontinence, loss of body wt and death) within 10–15 days. The disease produced here does not require inbred rat strains and occurs in female and male rats of the Upjohn Wistar and Carworth Sprague-Dawley strains. Lyophilized brain is incorporated also in the type of adjuvant (0.5 mg of *M. butyricum*; 0.1 ml of heavy mineral oil) employed routinely for the induction of arthritis. Although rats develop paralysis of a severe type with this latter material, they do not get polyarthritis.

Results. Given orally, both cyclophosphamide (Cytoxan) and U-29,409 inhibit the development of adjuvant-induced polyarthritis in rats (Table I). Both drugs are lethal at 16 mg/kg, bid, po. Cytoxan elicits antiarthritic effects at lower dosages than U-29,409. It requires 4 mg/kg of Cytoxan, and at least 8 mg/kg of U-29,409, to inhibit completely the development of arthritis. Cytoxan causes decreases in thymus weights; whereas, U-29, 409 fails to display similar inhibitory effects, at antiarthritic dosages. Spleen weights are decreased by both drugs. Testes weights are decreased only by U-29,409. However, the antiarthritic effects of U-29,409 are displayed equally well in female rats. The same dosages of both drugs to rats with well-established arthritis fail to inhibit the visual arthritic score. Aspirin, prednisolone, phenylbutazone, and indomethacin all produce dose-related inhibition of the score in rats with established arthritis (2).

It requires 5 days of Cytoxan and 15 days of U-29,409 therapy, at equivalent antiarthritic dosages, to elicit similar decreases in the arthritic score of rats with developing disease (Table II). Like previous results shown in Table I, therefore, U-29,409 is less effective than Cytoxan in the adjuvant-induced polyarthritis of rats.

When used as a combination, U-29,409 and Cytoxan appear to exert greater inhibitory effects on adjuvant-induced polyarthritis than either drug alone (Table III).

Both Cytoxan and U-29,409 inhibit the incidence of paralysis and the number of deaths in rats with allergic encephalomelitis (Table IV). Cytoxan is more effective than U-29,409.

When given orally to rats with carrageenan-induced acute hindpaw edema, U-29,409, unlike 6-alpha-methylprednisolone (Medrol) and aspirin, fails to inhibit this process (Table V). Both Cytoxan and U-29,

TABLE I. Comparison of U-29,409 and Cyclophosphamide in Treatment of Arthritic Rats.*

Drug	Dose (mg/kg, po, bid, ×15)	Body wt gain (g/rat)	Score, day 16	Organ wt (mg)			
				Thymus	Paraaortic nodes	Spleen	Testes
Vehicle (water)	—	−3	11.4 ± 1.3	407 ± 42	297 ± 22	1428 ± 118	3450 ± 70
Cyclophosphamide	1	+17	5.7 ± 1.5	—	—	—	—
	2	+20	2.2 ± 1.2	—	—	—	—
	4	+16	0 ± 0	203 ± 28	181 ± 30	632 ± 31	3490 ± 57
	10 (5/10 dead)	−57	0 ± 0	—	—	—	—
	16 (10/10 dead)	—	—	—	—	—	—
U-29,409	1	+5	11.2 ± 1.6	—	—	—	—
	2	+10	6.4 ± 1.2	—	—	—	—
	4	+11	5.6 ± 1.6	—	—	—	—
	8	+19	0 ± 0	376 ± 45	237 ± 22	755 ± 22	2150 ± 90
	16 (10/10 dead)						
No treatment (adjuvant only)		+4	13.2 ± 1.6				
Normal controls: no adjuvant		+73	—	741 ± 28	109 ± 16	867 ± 29	3710 ± 50

* Ten rats per group; ± = SEM; drugs started day before adjuvant (day 1) and given for 15 days.

TABLE II. Oral Dosages of Cyclophosphamide and U-29,409 Required for Inhibition of Adjuvant-Induced Polyarthritis in Rats.[a]

Drug	Administered, po, bid (days)	Body wt gain (g/rat), day 16	Score, day 16	Inhibition (%)
Cyclophosphamide, 8 mg/kg, bid	0	+ 7	12.3 ± 1.5	0
	0, 1	+14	10.8 ± 1.6	5
	0, 1, 2	+ 8	9.8 ± 1.6	14
	0, 1, 2, 3	+ 9	6.7 ± 1.3	41
	0, 1, 2, 3, 4	0	1.0 ± 1.0	91
	0, 1, 2, 3, 4, 5	+ 3	0 ± 0	100
	0, 1, 2, 3, 4, 5, 6	—25	0 ± 0	100
	0, 1, 2, 3, 4, 5, 6, 7	— 7	0 ± 0	100
	0 thru 15	—42	0 ± 0	100
U-29, 409, 8 mg/kg, bid	0	+13	11.1 ± 1.6	3
	0, 1	+ 6	12.5 ± 1.1	0
	0, 1, 2	+19	10.6 ± 1.6	7
	0, 1, 2, 3	+16	12.5 ± 1.6	0
	0, 1, 2, 3, 4	+14	11.9 ± 1.9	0
	0, 1, 2, 3, 4, 5	+21	9.7 ± 1.6	15
	0, 1, 2, 3, 4, 5, 6	+22	7.8 ± 1.6	32
	0, 1, 2, 3, 4, 5, 6, 7	+31	4.1 ± 1.6	64
	0 thru 15	+49 (3 dead)	0 ± 0	100
Vehicle (water)	0 thru 15	+24	11.4 ± 1.6	—
No treatment	1 only	+17	10.3 ± 1.6	—

[a] Ten rats per group; drugs given as shown starting on day 0; adjuvant given into tail, day 1.

TABLE III. U-29,409 Combined with Cyclophosphamide in the Prophylactic Treatment of AA.[a]

Drug	Dose (mg/kg, po, bid, × 15 days)	Body wt gain, day 15 (g/rat)	Arthritic score		
			Day 16	Day 21	(Day 21) Inhibition (%)
1. Vehicle	—	+13	6.1 ± 1.6	12.7 ± 1.1	0
2. Cyclophosphamide	1	+13	6.6 ± 1.6	9.8 ± 1.6	22.8
3.	2	+23	1.3 ± 0.8	5.9 ± 1.6	53.5
4. U-29,409	1	+ 2	11.3 ± 1.6	14.4 ± 1.6	0
5.	2	+ 3	10.4 ± 1.6	16.0 ± 0	0
6. 2 + 4	—	+ 6	4.8 ± 1.6	8.4 ± 1.6	33.9
7. 2 + 5	—	+18	2.0 ± 0.06	9.4 ± 1.6	26.8
8. 3 + 4	—	+14	0 ± 0	2.4 ± 0.08	81.0
9. 3 + 5	—	+29	0 ± 0	2.4 ± 1.6	81.0
10. No treatment	—	+ 6	11.6 ± 1.6	14.4 ± 1.6	0

[a] Ten rats per group; drugs started day 0; rats injected with *M. butyricum* in tail day 1; and drugs continued additional 14 days and withdrawn after 15 days of therapy.

TABLE IV. Effect of U-29,409 and Cyclophosphamide on Development of Allergic Encephalomyelitis in Female Rats.[a]

Treatment	Dose (mg/kg, po, bid, × 15 days)	No. of animals paralyzed; days after inoculation			
		10	12	14	16
Vehicle (water)	—	9/10 (1/10)[b]	7/7 (3/10)	3/3 (7/10)	1/2 (8/10)
U-29,409	6	0/10 (1/10)	1/9 (1/10)	2/8 (2/10)	2/7 (3/10)
Cyclophosphamide	6	0/10 (0/10)	0/10 (0/10)	0/10 (0/10)	0/10 (0/10)

[a] Both drugs killed all rats at 12 mg/kg. Carworth female rats were inoculated into the tail with 0.3 ml of Freund's complete adjuvant containing 6% lyophilized guinea pig brain.

[b] The number of animals dead, when examined on a particular day, is given in parentheses.

TABLE V. Effect of U-29,409 on Acute Carrageenan-Induced Hindpaw Edema of the Rat.[a]

Drug	Dose (mg/kg, po)	Edema (mg/100 g of body wt)	Inhibition (%)
Vehicle	—	255 ± 25	0
Medrol	2.5	198 ± 10	22
	5	140 ± 5	46
	15	80 ± 10	69
Aspirin	50	160 ± 25	38
	200	140 ± 10	46
	450	50 ± 11	71
U-29,409	50	270 ± 15	0
	200	250 ± 28	0
	450	248 ± 30	0

[a] Ten rats per group; ± = SD.

409, given twice each day for 7.5 days at effective antiarthritic dosages, fail to inhibit the carrageenan-induced inflammatory reaction performed on the eighth day.

When given at 100% inhibitory dosages for 2 weeks to rats with developing arthritis, both U-29,409 and Cytoxan depress the levels of circulating white cells (Table VI). Cytoxan has a greater inhibitory effect on lymphocytes; U-29,409 appears to exert its inhibitory effects, primarily, on granulocytes.

In rats with yeast-induced fevers, neither Cytoxan nor U-29,409 exerts inhibitory effects on elevated body temperatures. Aspirin, given at the same time, elicits dose-related decreases in body temperature.

In various *in vitro* systems (7) used for the assessment of standard nonsteroidal anti-inflammatory drugs, U-29,409 is inactive at concentrations of 10^{-3} *M*.

Summary and Discussion. U-29,409 (2,3-dihydro-(1-naphthyl)-4-(1*H*)-quinazolinone), a male antispermatogenic drug, exerts antiarthritic effects in rats similar to those elicited by Cytoxan. However, it is less effective than the latter. Laboratory screening tests fail to

TABLE VI. Effect of Cyclophosphamide and U-29,409 on Circulating White Cells of Rats with Developing Arthritis.[a]

Drug	Dose (mg/kg, po, bid, × 15 days)	Score, day 16	White cells/mm³			
			Total	Monocytes	Granulocytes	Lymphocytes
Vehicle	—	11 ± 1.6	25,540 ± 890	1772 ± 136	12,940 ± 655	10,498 ± 617
Cyclophosphamide	4	0	9360 ± 535	626 ± 55	7333 ± 420	1352 ± 142
U-29,409	8	0	8565 ± 880	286 ± 70	1947 ± 235	6245 ± 394
Normal rats	—	0	9445 ± 580	324 ± 49	860 ± 122	8180 ± 572

[a] Ten rats per group; ± = SEM. Drugs started day 0; *M. butyricum* inoculated day 1; rats exsanguinated day 16.

show that U-29,409 has acute anti-inflammatory effects when given as a single dose or for 1 week prior to performing acute inflammatory reactions. In combination with Cytoxan, U-29,409 appears to exert a synergistic inhibitory effect on developing arthritis. Neither Cytoxan nor U-29,409 appears to exert significant inhibitory effects in rats with established disease. Both drugs, unlike non-steroidal anti-inflammatory drugs, fail to display inhibitory effects in yeast-induced fever of rats.

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