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Inhibition of Adjuvant-Induced Polyarthritis with Cytarabine (33443)

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Adjuvant-induced polyarthritis is thought to be a "delayed hypersensitivity" disease (1, 2). It is characterized by proliferative joint disease and a variety of other clinical features. It has many features characteristic of Reiter's disease in man (3). Various workers (4–7) have shown inhibition of the disease with so-called immunosuppressants and cytotoxic drugs. Nonsteroidal and steroidal anti-inflammatory drugs inhibit adjuvant-induced polyarthritis differently. The nonsteroidal anti-inflammatory drugs ameliorate the systemic inflammatory component; without altering the course of the disease. The steroidal drugs inhibit the onset, severity, and progression of the disease by depressing the reactivity of the draining lymph nodes primarily and the inflammatory phases of the systemic disease secondarily (7).

Cytarabine depresses bone marrow function, inhibits antibody formation and is cytotoxic to various cells when added *in vitro* (8–11). Its effects on adjuvant-induced polyarthritis of rats were studied and these form the basis of the present report.

Methods. Adjuvant arthritis was produced by the inoculation of 0.5 mg of *M. butyricum*[1] in 0.1 ml of mineral oil directly into the tail of Sprague-Dawley male rats of the Badger colony (12–18). Animals usually develop arthritis within 14–20 days and are either scored visually and/or the acute phase reactants in the serum (19) are studied simultaneously; in order to ascertain the inhibitory effects of drugs.

The various other procedures (granuloma pouches, antipyretic and antihindpaw edema assays) have been summarized (20). Cytarabine and the other drugs were given as indicated in the Tables I–VIII.

Results. Cyclophosphamide and cytarabine do not possess local anti-inflammatory effects when injected directly into the granuloma pouches of rats. Hydrocortisone, used as additional controls in these studies, inhibits exudate formation in granuloma pouches (Table I). 5-Fluorouracil and 2-amino-6-mercaptopurine, but not 6-mercaptopurine, inhibit inflammatory exudate formation into granuloma pouches.

When given orally, cyclophosphamide and phenylbutazone depress the elevated temperatures of rats with yeast-induced fevers, but cytarabine shows no activity (Table II).

[1] Phenylbutazone was obtained from Geigy; Medrol from Upjohn; Cyclophosphamide from Mead Johnson; and *M. butyricum* from Difco.

TABLE I. Effect of Drugs on Inflammatory Exudate when Injected Locally.[a]

Drug	Dose (μg/pouch/day; × 7 days)	BW gain (g)	Exudate (ml)	Inhibition (%)
Vehicle	—	51 ± 2	19 ± 1.7	—
Cytarabine	100	63 ± 5	17 ± 2.8	0
	200	63 ± 3	20 ± 2.0	0
	400	59 ± 4	24 ± 1.7	0
Cyclophosphamide	100	60 ± 2	20 ± 1.0	0
	200	53 ± 3	21 ± 0.8	0
	400	58 ± 4	20 ± 1.3	0
Hydrocortisone	200	45 ± 3	8 ± 1.2	58
	400	55 ± 7	2 ± 0.7	88
5-Fluorouracil	200	55 ± 2	13 ± 2	38
6-Mercaptopurine	200	48 ± 7	19 ± 2	0
2-Amino-6-mercaptopurine	200	44 ± 3	6 ± 1	72
Hydrocortisone	200	39 ± 0.2	4 ± 1	79
Vehicle	—	44 ± 2	21 ± 1	—

[a] Ten rats/group; upper part of table represents one experiment and lower part represents another; ± SEM in this and all other tables; and BW = Body weights in this and all other tables.

Cytarabine is ineffective in the hindpaw edema assay of rats; cyclophosphamide is effective at relatively high dosages and phenylbutazone exerts the expected effects when tested simultaneously (Table III). When given intraperitoneally, however, (3–40 mg/kg) both immunosuppressants are inactive in this test. Cytarabine (40 mg/kg) was given intraperitoneally for 8 days and the hindpaw edema assay was performed on the last day of therapy. Although the polymorphonuclear leukocyte count was reduced

TABLE II. Effect of Various Drugs on Elevated Body Temperatures of Rats.[a]

Drug	Dose (mg/kg, p.o.)	Drop in body temp. (°F) 2 hr	4 hr	6 hr
Vehicle	—	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
Cytarabine	6	0	0	0
	12	0	0	0
	24	0	0	0
	48	0	0	0
	96	0	0	0
Phenylbutazone	12	1.3 ± 0.04	1.0 ± 0.1	0.6 ± 0.1
	92	3.0 ± 0.1	3.2 ± 0.09	3.0 ± 0.1
Vehicle	—	0.1 ± 0.1	0.2 ± 0.4	0.7 ± 0.2
Cyclophosphamide	25	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.2
	50	1.0 ± 0.1	0.5 ± 0.1	0.8 ± 0.1
	100	1.2 ± 0.2	0.9 ± 0.3	0.8 ± 0.3
Phenylbutazone	48	1.8 ± 0.1	2.1 ± 0.2	1.9 ± 0.1

[a] Ten rats/group; animals injected with 400 mg of yeast at 4 p.m.; those with body temperatures in excess of 101°F were selected for above experiments. Upper part of table, one experiment; lower part, another.

TABLE III. Effect of Various Drugs on Hindpaw Edema of the Rat.[a]

Drug	Dose (mg/kg, p.o.)	Edema (mg/100 g of BW)	Inhibition (%)
Vehicle	—	372 ± 25	—
Cytarabine	8	406 ± 8	0
	16	328 ± 20	13
	32	364 ± 27	0
	64	377 ± 26	0
Phenylbutazone	8	282 ± 10	25
	16	264 ± 11	30
	32	232 ± 8	38
	64	204 ± 18	46
Cyclophospha-mide	8	379 ± 10	0
	16	416 ± 21	0
	32	347 ± 13	7
	64	255 ± 11	32

[a] Ten rats/group.

to 26% of controls, cytarabine failed to inhibit the acute inflammatory reaction of the hindpaw induced by carrageenin.

When given orally, cytarabine is ineffective in the treatment of arthritic rats (Table IV); while phenylbutazone and cyclophosphamide depress the clinical disease and the increased fibrinogen and plasma inflammation units (19, 20). When given intraperitoneally, cytarabine is an effective antiarthritic drug. It appears to be somewhat less toxic than cyclophosphamide given by the same route (Table V).

Cytarabine has no effect on arthritis once the disease is well established. It must be given for at least 4–8 days during the development phases before significant inhibitory effects on arthritis can be had (Table VI).

When arthritis is inhibited completely by cytarabine, it fails to reappear following cessation of therapy (Table VI). When given at low doses; in combination with low doses of 6-α-methyl-prednisolone (Medrol), both drugs exert greater inhibitory action on the arthritic incidence than either drug alone (Table VII).

Cytarabine induces "tolerance" to further *M. butyricum* inoculations when given at effective doses throughout the 2-week induction phase of the disease (Table VIII). However, when given daily for 2 weeks before the inoculation of *M. butyricum* into the tail, it

TABLE IV. Effect of Phenylbutazone, Cyclophosphamide and Cytarabine on Adjuvant Arthritis when Given Orally.[a]

Drug	Dose (mg/kg, p.o.; b.i.d. × 14 days)	BW gain (g)	Incidence	Score	Inhibition (%)	Fibrinogen (mg/100 ml)	Inhibition (%)
Vehicle (water)	—	39 ± 5.1	10/10	13 ± 0.8		1521 ± 80	—
Phenylbutazone	4	53 ± 3.8	10/10	8 ± 0	38		
	8	71 ± 4.2	10/10	6 ± 0.7	54		
	16	75 ± 7.2	9/10	5 ± 0.9	62		
	33	67 ± 7.0	8/10	3 ± 0.6	77		
	68	72 ± 8.6	7/10	3 ± 0.7	80	742 ± 89	52
Cytarabine	4	40 ± 9	10/10	12.7 ± 0.8	0		
	9	43 ± 6.6	10/10	11.6 ± 0.9	0		
	17	42 ± 4.9	10/10	12.4 ± 0.8	0		
	37	34 ± 6.1	10/10	12 ± 0.9	0		
	72	42 ± 4.5	10/10	7.7 ± 0.6	41	1448 ±108	0
Cyclophosphamide	0	44 ± 6.4	10/10	10 ± 1.1	23		
	1	42 ± 6.6	8/10	5 ± 0.9	62		
	3	16 ± 7.3	2/10	0.2 ± 0.2	98		
	7	26 ± 5.2	6/10	2.2 ± 0.4	83	652 ± 64	57
	16	All dead					

[a] Ten rats/group; *M. butyricum* (0.5 mg/0.1 ml of mineral oil) inoculated into tail Day 0; drugs started orally, 2×/day, on Day 1.

TABLE V. Effect of Cyclophosphamide and Cytarabine on Adjuvant-Induced Polyarthritis when Given Intraperitoneally.[a]

Drug	Dose (mg/kg, i.p.; b.i.d. × 14 days)	BW gain (g)	Incidence	Arthritic score	Inhibition (%)	Plasma inflammation (units)	Inhibition (%)
Vehicle	—	31 ± 6	10/10	12.2 ± 1	—	78 ± 3	
Cytarabine	1.1	22 ± 3	10/10	10.6 ± 1	13	77 ± 2	0
	2.2	33 ± 5	9/10	7.7 ± 1.5	37	61 ± 5	20
	4.4	39 ± 6	8/10	5.8 ± 1	53	62 ± 3	20
	8.8	46 ± 5	7/10	3.8 ± 1	69	70 ± 3	10
	16.6	64 ± 3	1/10	3.4 ± 0.4	97	44 ± 4	44
	33.2	56 ± 6	0/10	0 ± 0	100	25 ± 3	68
Cyclophosphamide	0.4	44 ±10	10/10	8.6 ± 1	30	50 ± 4	36
	0.8	45 ± 8	9/10	8.8 ± 1	28	69 ± 3	12
	1.6	52 ± 4	9/10	5.0 ± 1	60	55 ± 3	30
	3.2	64 ± 3	3/10	0.9 ± 0.3	93	28 ± 4	65
	6.4	30 ± 6	2/10	0.4 ± 0.2	96	25 ± 1	68
	12.8 (4/10 dead)	−28 ± 6	0/6	0 ± 0	100	42 ± 7	47

[a] Ten rats/group; *M. butyricum* (0.5 mg/0.1 ml of mineral oil) into tail Day 0; drugs started Day 1 and administered intraperitoneally 2×/day × 14 days; animals scored and incidence of arthritis made by disinterested observer.

TABLE VI. Effect of Various Dosage Regimens of Cytarabine on Adjuvant-Induced Polyarthritis.[a]

Drug	Dose (mg/kg, i.p.; b.i.d.)	Treatment (days)	BW gain day 0–15	Incidence Day 16	Incidence Day 28	Score Day 16	Score Day 28
Vehicle	—	15	28	9/10	8/9	13.0	11.5
Cytarabine	40	1	22	10/10	10/10	12.0	11.6
	40	2	32	9/9	9/9	13.0	12.1
	40	3	42	10/10	10/10	9.5	8.9
	40	4	59	7/10	7/10	7.6	6.3
	40	8	91	1/10	1/10	0.2	0.2
	40	12	89	0/10	0/10	0	0
	40	16	64	0/10	0/10	0	0

	Dose (mg/kg, i.p.; b.i.d. × 14 days)	BW gain day 15–29	Arthritic score Initial day 15	Arthritic score Final day 29	Inhibition (%)
Vehicle	—	10 ± 3	14.3	13.8 ± 0.4	
Cytarabine	17	7 ± 6	15.2	14.2 ± 0.5	0
	32	4 ± 3	14.7	14.3 ± 0.5	0
	70	−5 ± 5	14.5	13.2 ± 0.8	0

[a] Upper part of table represents experiment in which rats were dosed with drug at times indicated; lower part represents treatment for 2 weeks after the development of fully established disease; 10 rats/group.

TABLE VII. Inhibitory Effects of Medrol and Cytarabine on Incipient and Established Arthritis of Rats.[a]

Drug	Dose (mg/kg/day)	Therapy (days)	Score day 15	Incidence day 15
Vehicle		1–14	14.1 ± 0.8	10/10
Medrol	0.5 (s.c.)	1–14	3.3 ± 0.6	9/10
Cytarabine	20.0 (i.p.)	1–14	7.8 ± 1.2	9/10
Medrol + cytarabine	0.5 + 20.0	1–14	1.4 ± 0.8	3/10

			Score		Incidence	
			Day 15	Day 28	Day 15	Day 28
Vehicle		15–28	19.9 ± 0.3	13.7 ± 0.2	10/10	10/10
Medrol	1.0 (s.c.)	15–28	13.5 ± 0.1	12.6 ± 0.6	10/10	10/10
Cytarabine	38.0 (i.p.)	15–28	13.6 ± 0.2	13.6 ± 0.3	10/10	10/10
Medrol + cytarabine	1.0 + 38.0	15–28	13.1 ± 0.1	7.9 ± 1.4	10/10	6/10

[a] Ten rats/group; upper part of table represents experiments in which drugs were given during incipient phases of disease; lower part represents those in which drugs were given to rats with established disease.

TABLE VIII. Induction of Tolerance by Pre-treatment of *M. butyricum* Inoculated Rats with Cytarabine.[a]

Treatment	Dose (mg/kg), i.p.; b.i.d. × 14 days)	Arthritic incidence and score		
		Day 15	Day 49	Day 71
Vehicle	—	12.0 ± 0.6 (16/17)	8.0 ± 0.5 (16/17)	9.0 ± 0.7 (16/17)
Cytarabine	30	0 (0/20)	0 (0/20)	0 (0/20)

[a] Rats inoculated with *M. butyricum* (0.5 mg/0.1 ml of mineral oil) on Days 0 and 49; rats were treated with cytarabine from Day 1–14 and treatment discontinued thereafter; nos. in parentheses represent number of rats with arthritis.

does not inhibit the development of arthritis 2 weeks later.

Discussion and Summary. Cytarabine, like other cytotoxic drugs, inhibits the onset of adjuvant-induced polyarthritis. Both steroidal and nonsteroidal "anti-inflammatory" drugs inhibit the severity of adjuvant-induced polyarthritis (6, 7, 20). The steroids are more effective, since they inhibit not only the onset and development of the disease, but also ameliorate its symptoms after it becomes well established. The current nonsteroidal anti-inflammatory drugs are less effective than steroids on all parameters.

Cytarabine, when compared to cyclophosphamide and phenylbutazone, has no "anti-inflammatory" and antipyretic activity. It inhibits the draining lymph node hypertrophy which occurs after the inoculation of *M. butyricum* into the tail (19). Therefore, it may be added to the list of available cytotoxic drugs called "immunosuppressants."

Cytarabine is not effective at the doses used when given orally. It must be given parenterally. Cyclophosphamide and Medrol are effective by both routes.

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Estradiol Antagonism of Chlorpromazine Hyperglycemia* (33444)

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Several investigators have reported the antidiabetic effect of the female sex hormone. Nelson and Overholser (1) have shown that estrone reduces hyperglycemia and glycosuria in monkeys administered pituitary extract. Estradiol propionate in rabbits (2) and in women (3) have been shown to decrease hyperglycemia, and Rodriguez (4) has shown that about 50% of alloxan-diabetic rats treated with estradiol maintain normal blood sugar levels.

In a previous publication (5) we reported the interaction of estradiol and chlorpromazine with respect to the metabolism of the latter. This report on the antagonism of chlorpromazine hyperglycemia by estradiol is submitted as a portion of our continuing studies on the interaction of hormones with drugs.

Materials and Methods. Male albino Holtzman rats weighing approximately 200 g were employed. Estradiol valerate in sesame oil 25 μg/rat, s.c., were administered on day 0 and day 5. Three days after the second injection the animals were fasted 16–20 hr with water *ad libitum* prior to chlorpromazine administration. Groups of 5 treated and control rats were administered chlorpromazine 10 mg/kg, i.p. or glucose 2 gm/kg, i.p. and blood glucose was determined by the glucose oxidase method [2] on blood obtained by the orbital sinus technique (6).

In the second experiment, rats were treated with estradiol as previously described. The diaphragms were removed and incubated in 10 ml of Krebs-Hensleit solution containing 2 g/liter glucose in a shaker incubator at 37° using oxygen. Disappearance of glucose from the media was followed at various time intervals and is reported as milligrams of glucose disappearance per gram of tissue. Determinations were made with 5 mg chlorpromazine added as well as with untreated rat diaphragms.

Results and Discussion. As shown in Fig.

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[2] Glucostat, Worthington Biochemical Co.