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The Teratogenic Effects of 5-Fluorocytosine in the Rat[1]

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SUMMARY

Single i.p. injections of 5-fluorocytosine (5-FC) at doses ranging from 500 to 4000 mg/kg of maternal body weight given to pregnant Wistar rats on Days 9–12 of gestation produced gross malformations which included cleft palate, cleft lip, deformed appendages, micrognathia, and short kinky tails in fetuses from rats treated only on Day 11 or 12 which survived to Day 21 of gestation. No malformations were observed in fetuses at 21 days with any of the doses (500–4000 mg/kg) given to the pregnant rat on Day 9 or 10 of gestation. 5-FC was in the range of 2416–9000 times less effective in producing malformations in the 12-day fetal rat than its deoxyriboside, 5-fluorodeoxycytidine. The types of malformations produced by the two compounds, however, were similar.

INTRODUCTION

5-Fluorocytosine (5-FC)[3], an antimetabolite of cytosine, was synthesized by Duschinsky *et al.* (5). Unlike its riboside and deoxyriboside (2), 5-FC was inactive against transplantable rodent tumors (7) and leukemias (9) and lacked bacteriocidal activity *in vitro* (5). It was, however, significantly active *in vitro* (10) and *in vivo* (6, 10) against experimental systemic *Candida albicans* in mice. In preliminary clinical trials, 5-FC showed therapeutic activity in the treatment of moniliasis in man[4].

The present report describes the malformations produced in the 21-day-old fetus when 5-FC was given to pregnant rats on Days 9–12 of gestation.

MATERIALS AND METHODS

One hundred and one female rats of the CF Wistar strain, weighing from 200 to 250 gm, were mated during estrus by exposure to males of the same strain. The day on which sperm were found in vaginal smears was considered as Day 1 of gestation. Pregnant females were caged separately and fed on Purina chow and oatmeal with water *ad libitum*. On a day selected for treatment, from the 9th to the 12th day of gestation, the rats were injected with single i.p. doses of 5-FC on a mg/kg basis of maternal body weight. The controls received the vehicle, carboxymethylcellulose. The animals were sacrificed on Day 21 of gestation (the day before expected littering) and the live fetuses removed, weighed, and examined for gross malformations. A selected number from each litter was fixed in 95% ethanol for subsequent staining with alizarin red for examination of the bony skeleton (4). The number of resorbed and dead fetuses in each litter was also recorded.

5-FC was suspended in 0.5% carboxymethylcellulose and prepared freshly on each injection day.

RESULTS

Maternal Lethality. Single i.p. injections of 500–2000 mg/kg of 5-FC given to pregnant rats from Day 9 to 12 of gestation were not lethal, but doses of 3000 and 4000 mg/kg killed 50% (estimated maternal LD_{50} dose) of the mothers treated on Day 9 or 11 and 10 or 12 respectively by the 21st day of gestation.

Fetal Effects of Single Doses of 5-FC. The effects of single doses of 5-FC on the rat fetus when pregnant rats were treated once on Days 9–12 of gestation is shown in Chart 1. The lowest dose of 500 mg/kg was not lethal to the fetus on any of the 4 treatment days, but increasingly higher doses of the drug (700–4000 mg/kg) killed greater numbers of fetuses (% fetal mortality) by the 21st day. Total destruction of litters was observed with a dose of 4000 mg/kg of 5-FC given to pregnant rats on Day 9, 10, or 11 of gestation. Single doses of 5-FC ranging from 500 to 3000 mg/kg injected into pregnant rats on Day 9 or 10 of gestation did not produce any malformations in fetuses that survived to Day 21, nor were malformations observed in fetuses from rats treated with 500 mg/kg on Day 11 or 12. The lowest teratogenic dose was 700 mg/kg (Days 11 and 12) and the highest 4000 mg/kg (Day 12). Within this dose range, single injections of 1000, 2000, 3000 and 4000 mg/kg given to the rat on Day 11 or 12 of gestation produced increasingly greater numbers of abnormal survivors at 21 days.

Representative fetuses from rats treated with 5-FC on Day 11 or 12 of gestation are shown in Fig. 1, and the types and incidence of selected malformations are summarized in Table 1. Gross malformations include deformed appendages, paws, and tails and cleft palate on Days 11 and 12, additionally, cleft

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[3]Samples of 5-fluorocytosine were kindly supplied by Dr. Edward Miller of Hoffmann-LaRoche, Inc., Nutley, N. J.

[4]Clinical Data Sheet on 5-fluorocytosine (RO-29915), Hoffmann-LaRoche, Inc., Nutley, N. J.

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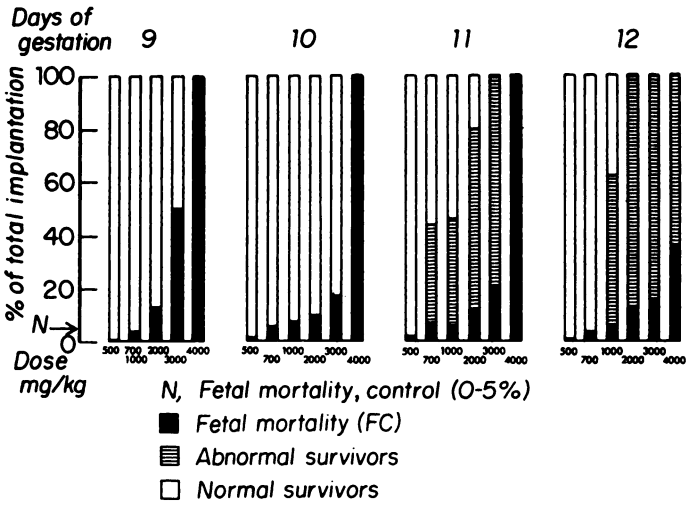

Chart 1. The lethal and teratogenic effects of 5-fluorouracil in rats (single i.p. injections into pregnant rats on Days 9–12 of gestation; rats were sacrificed on Day 21 of gestation).

lip on Day 11. Skeletal defects include absence of ossification of bones of the skull, short or absent limb bones, and incompletely ossified vertebrae (Fig. 2).

The teratogenic activity of the deoxyriboside of 5-FC, 5-fluorodeoxycytidine (FCdR), has been demonstrated previously (3). Although the types of malformations produced by 5-FC were similar to those produced by FCdR, the doses required were from 2416 to 9000 times greater (Table 2).

DISCUSSION

These studies have demonstrated that single i.p. injections of 5-FC at doses ranging from 700 to 4000 mg/kg, given to pregnant rats on Days 9–12 of gestation, were lethal to varying degrees on all 4 treatment days. Such treatment produced malformations in fetuses surviving to the 21st day only from rats treated on Day 11 or 12 of gestation at doses which were about 4–5 times lower than the doses which were lethal to the adult (700 mg/kg on Day 11 or 12 *vs* 3000 and 4000 mg/kg, the estimated maternal LD_{50} doses, on Day 11 and 12 respectively).

Toxicity studies have shown that 5 daily injections of 1000 mg/kg of 5-FC produce no toxic effect in the rat[5], whereas 5-fluorouracil (5-FU) given in a similar manner was lethal at 25 mg/kg/day × 5 to half the number of treated rats (LD_{50})[6]. In view of the relative lack of toxicity of 5-FC, it seems unlikely that it would be deaminated to any appreciable extent to 5-FU. A similar reasoning can also be used against *in vivo* conversion, if any, of 5-FC to the nucleoside or nucleotide since they are extremely toxic in the rat, e.g., the LD_{50} of FCdR in the rat is 5 mg/kg/day × 5 (6).

The mechanism by which 5-FC produces developmental defects in the rat fetus cannot be adduced from these experiments. Evidence for the presence of unchanged pyrimidine, obtained by paper chromatograms of samples of urine collected from rats treated with 5-FC (100 mg/kg, i.p. dose) and radioactive 5-FC-2-^{14}C (14.5 mg/kg, i.p. dose), show that about 62.3% and 60.2% respectively of the injected doses of the drug are excreted within 12–24 hours after injection (8). Cytosine was similarly found to be relatively stable and was excreted unchanged in the urine in rats (1). It appears, therefore, that both 5-FC and cytosine are relatively resistant to biotransformation in the rat.

[5]F. S. Philips, A. P. Cronin, and P. M. Vidal. Observations on 5-Fluorocytosine. Informal Report, The Sloan-Kettering Institute for Cancer Research, New York, N.Y., March 30, 1962.

[6]F. S. Philips, and S. S. Sternberg. Notes on the Toxicity of 2′-Deoxy-5-Fluorocytidine and of other 5-Fluoropyrimidines. Informal Report, The Sloan-Kettering Institute for Cancer Research, New York, N. Y., December 1, 1959.

Table 1

	Day of gestation								
	11				12				
	700[a]	1000	2000	3000	700	1000	2000	3000	4000
% fetal mortality	7	6	12	20	N[b]	N	13	16	36
Abnormal survivors/total survivors	19/48	24/55	27/35	24/24	22/42	31/51	44/44	45/45	19/19
Number with selected abnormalities									
Retarded and/or clubbed									
Fore leg	0	0	14	10	21	11	44	45	19
Rear leg	0	0	11	13	22	16	44	45	19
Ectro-, syn-, brachy-, or Polydactylous									
Fore paw	0	0	5	12	22	20	44	45	19
Rear paw	0	0	22	19	22	12	44	45	19
Cleft palate	8	9	18	11	22	11	44	45	19
Cleft lip	0	0	4	8	0	0	0	0	0
Micrognathia	0	0	6	12	11	10	44	45	19
Kinked, short tail	0	0	12	14	21	17	44	45	19

Types and incidence of malformations observed in 21-day-old rat fetuses following single i.p. injections of 5-fluorocytosine into rats on the 11th or 12th day of pregnancy.

[a]Dose, mg/kg.

[b]Fetal mortality within the control range of 0–5%.

Table 2

	Estimated maternal LD_{50} dose, mg/kg[a] (mmoles/kg)	Estimated minimal fetal LD_{100} dose[b] mg/kg (mmoles/kg)	Teratogenic dose and range mg/kg (mmoles/kg)	Selected malformations within the teratogenic dose range					
				Cleft palate and lip	Retarded and/or clubbed		Syn-, ectro-, or polydactylous		Short kinked tail or tail absent
					Fore leg	Rear leg	Fore leg	Rear leg	
5-FC	4000 (31.0)	4000 (31.0)	700–3000 (5.4–23.2)	+	+	+	+	+	+
FCdR	>2000[c] (>8.1)	5 (0.0192)	0.15–2.5 (0.0006–0.0096)	+	+	+	+	+	+

A comparison of the effects of 5-fluorocytosine (5-FC) and 5-fluoro-2'-deoxycytidine (FCdR) in the rat (single i.p. injections into pregnant rats on Day 11 of gestation; rats sacrificed on Day 21 of gestation).

[a] Estimated maternal LD_{50} dose is the single dose given to the 11-day-pregnant rat that killed 50% of the treated mothers by Day 21 of gestation.

[b] Estimated minimal fetal LD_{100} dose is the single dose given to the 11-day-pregnant rat that killed 100% of the fetuses by Day 21 of gestation.

[c] This dose was tested in nonpregnant rats weighing 80 gm.

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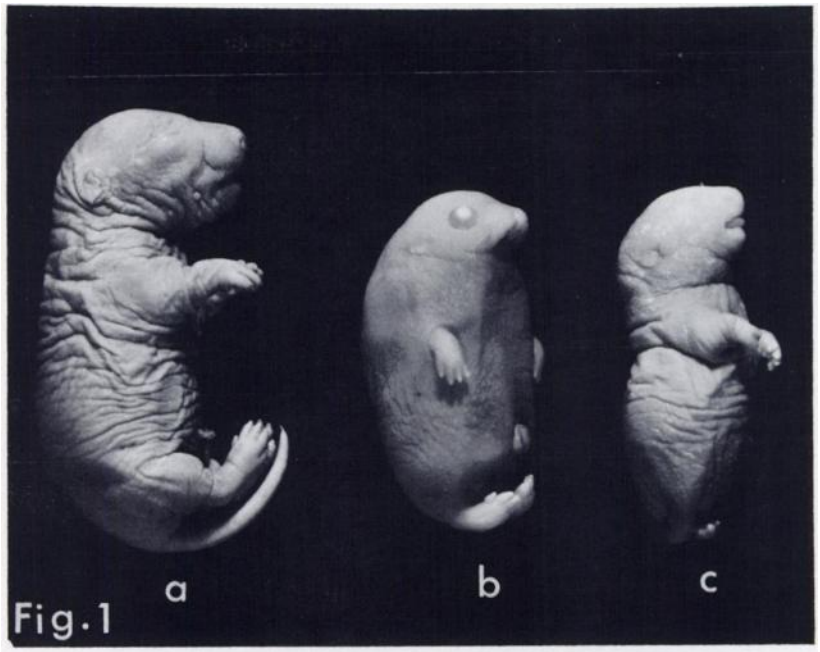

Fig. 1

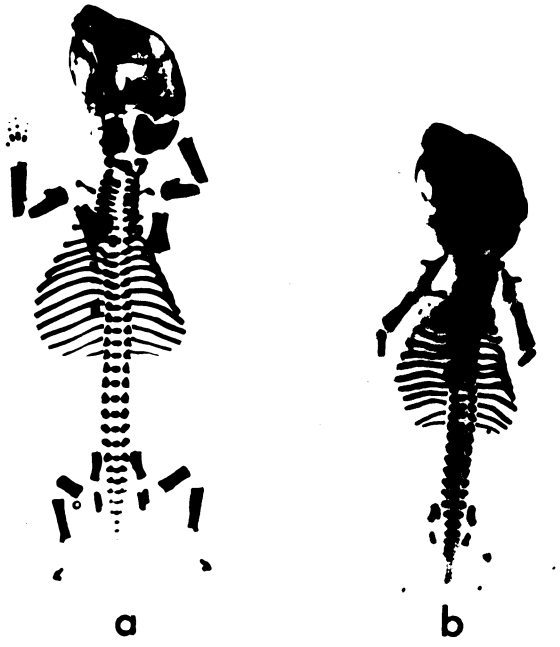

Fig. 2

Fig. 1. Fetuses 21 days old from rats treated with 5-fluorocytosine (5-FC). *a*, control; *b*, fetus from a rat given a single injection of 2000 mg/kg of 5-FC on Day 11 of gestation; *c*, fetus from a rat given a single injection of 1000 mg/kg of 5-FC on Day 12 of gestation. Both *b* and *c* have retarded and malpositioned appendages, deformed paws, short mandibles, and no tails; additionally *b*, has open eyes and cleft lip.

Fig. 2. Skeleton of 21-day-old fetuses stained with alizarin red. *a*, control, *b*, fetus from a rat given a single injection of 2000 mg/kg of 5-fluorocytosine on Day 12 of gestation, showing absence of femurs, fibulae, tibae, metatarsals, and ulnae, short humerii and radii, and retarded cervical vertebrae.