Cysteine Interference with Chlorambucil-Induced Teratogenesis in the Rat[1] (36077)

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It is a well-established hypothesis that as many as 60% of all human congenital abnormalities may be caused by an interaction between: (i) the environment and the genome; or (ii) two or more environmental factors (1). Because of the difficulties involved in experimental manipulation of human pregnancies, most work has been done with lower mammalian forms in which various combinations of exogenous drugs or chemicals have been tested to determine their combined effects on fetuses. Needless to say, numerous examples of chemical interaction have resulted (2–5). Only a few of these reports, however, have produced data suggesting that certain drugs interfere with or protect against teratogenesis induced by other agents (6–9).

Chlorambucil, an alkylating agent and potent teratogen (10, 11), is thought to have biological effects that are radiomimetic (12). It thus became of interest to determine if chemicals that are known to protect against the lethal or growth retarding effects of X-irradiation (13) also could inhibit abnormal development in embryos exposed to a radiomimetic teratogenic insult administered during the critical stages of gestation.

Materials and Methods. Pregnant Long-Evans black-hooded rats were divided into 5 groups. Animals from one group were given single, intraperitoneal injections of chlorambucil in sesame oil at a dose of 6 mg/kg on days 12, 13, or 14 of gestation (day 0 was considered to begin on the morning that sperm were found in the contents of a vaginal smear). Animals from another group were given an intraperitoneal injection of cysteine dissolved in distilled water at doses of 50, 100, 150, or 200 mg/kg 30 min before being exposed to chlorambucil on day 12. The remaining 3 sets of animals were controls that either were left untreated or given sesame oil or one of the 4 doses of cysteine alone on day 12. On day 20 of gestation (1 day before birth), the females were killed by cervical dislocation and the fetuses were removed, counted, and fixed in either Bouin's fluid or ethanol for subsequent gross or skeletal examination.

Results and Discussion. The number of pregnant rats receiving the various treatments and the resulting incidences of living and normal fetuses are shown in Table I. The percentages of living fetuses 8 days after treatment with sesame oil or cysteine at doses of 50 and 150 mg/kg did not differ significantly from the untreated control nor from one another as indicated by the chi-square test corrected for continuity ($p<.05$) (14). However, treatment with 100 or 200 mg/kg of cysteine alone resulted in a significant decrease in the percentage of living offspring. Of the surviving fetuses from the untreated control, sesame oil, and the four cysteine groups, nearly all were structurally normal.

After exposure on day 12 to chlorambucil alone or in combination with different doses of cysteine, the percentages of living fetuses were significantly less than most of the corresponding controls, though not different from one another. Chlorambucil administered on days 13 or 14 did not result in a difference in

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TABLE I. Incidence of Living and Normal Fetuses.[a]

Treatment	Dose (mg/kg)	No. of females	No. of implantation sites	Living		Normal	
				No.	(%)	No.	(%)
Untreated control	—	28	319	314	(98.4)	310	(98.7)
Sesame oil[b]	—[c]	10	104	101	(97.1)	99	(98.0)
Cysteine[b]	50	4	42	40	(95.2)	40	(100)
	100	5	58	49	(84.5)	49	(100)
	150	4	55	55	(100)	55	(100)
	200	4	33	30	(90.9)	29	(96.7)
Chlorambucil[b]							
Day 12	6	32	374	310	(82.9)	34	(11.0)
13	6	5	60	59	(98.3)	9	(15.3)
14	6	19	185	184	(99.5)	16	(8.7)
Cysteine	50 + 6	6	68	58	(85.3)	28	(48.3)
+ Chlorambucil[d]	100 + 6	6	74	61	(82.4)	38	(62.3)
	150 + 6	13	149	129	(86.6)	65	(50.4)
	200 + 6	7	77	61	(79.2)	15	(24.6)

[a] Pregnant females killed on day 20 of gestation and the fetuses examined for gross and skeletal defects.

[b] A single, intraperitoneal injection on day 12 of gestation or on day specified.

[c] Volume comparable to chlorambucil injection (2 ml/kg).

[d] Consecutive intraperitoneal injections separated by a 30 min time interval on day 12 of gestation.

the percentages of living young when compared to the controls. The rates of normality in the surviving fetuses were significantly decreased when the animals were given chlorambucil alone on days 12, 13, or 14, whereas previous treatment with cysteine resulted in a significant increase in the percentage of normal survivors when compared to chlorambucil alone. Moreover, the apparent protection that cysteine afforded the fetuses appeared dose dependent. Though cysteine at 100 mg/kg increased the percentage of normal young by approximately 6-fold, the proportion of abnormal fetuses still remained somewhat greater than the corresponding controls.

To determine the degree of cysteine protection, the percentages of fetuses having one or more structural abnormalities were compared (Table II). Chlorambucil alone on day 12 induced 3 or more anomalies in 48.1% of the living young and lower percentages of offspring suffering from fewer defects. With cysteine at doses of 50, 100, or 150 mg/kg, a majority of the abnormal fetuses suffered from only 1 malformation; whereas the highest and least protective dose of cysteine (200 mg/kg) resulted in a distribution of abnormalities resembling the pattern caused by the teratogen alone.

The mechanism by which cysteine protects against chlorambucil-induced teratogenesis, the basis for the decrease in relative severity of anomalies, and the site of protection are

TABLE II. Percentage of Offspring Having One or More Structural Defects.[a]

Treatment	No. of defects		
	1	2	3 or more
Chlorambucil[b]	20.8	31.1	48.1
Chlorambucil[c]			
+ cysteine, 50 mg/kg	80.0	6.7	13.3
100 mg/kg	60.9	21.7	17.5
150 mg/kg	64.1	15.6	20.2
200 mg/kg	13.0	21.7	65.3

[a] Pregnant females were killed on day 20 of gestation and the fetuses were examined for gross and skeletal defects.

[b] A single, intraperitoneal injection on day 12 of gestation.

[c] Consecutive intraperitoneal injections separated by a 30 min time interval on day 12 of gestation.

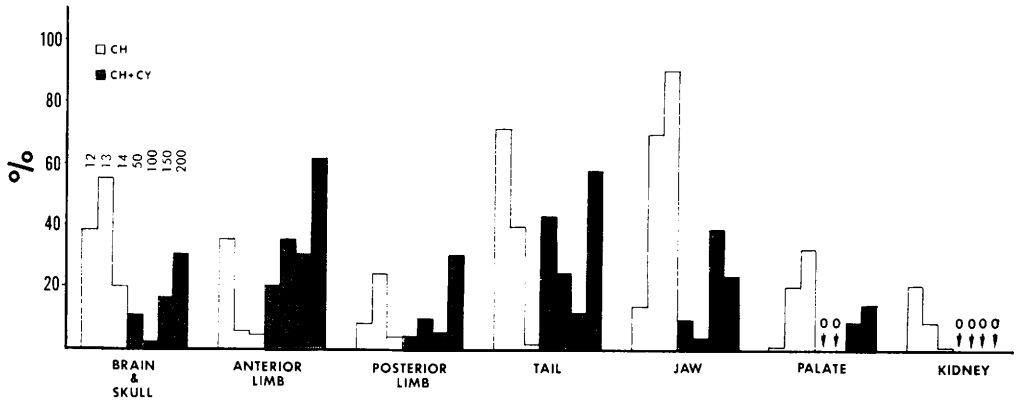

FIG. 1. The percentages of abnormal fetuses having particular malformations: CH, chlorambucil administered on day 12, 13, or 14 of gestation at a dose of 6 mg/kg; CH+CY, a single injection of chlorambucil on day 12 at a dose of 6 mg/kg preceded by a single injection of cysteine 30 min before at doses of 50, 100, 150, or 200 mg/kg. Brain and skull defects included hydrocephaly and meningoencephalocele; anterior limb abnormalities consisted of syndactyly, phocomelia, and hemimelia; posterior limb defects included syndactyly, polydactyly, hemimelia, and clubfoot; tail anomalies were composed of short or kinky tails; jaws were micrognathic; palates were cleft; and kidneys were horseshoe, small, and absent.

not yet clear. Alkylating agents are known to rapidly react with nucleophilic centers including thiols, carboxyl groups, phosphates; the ring nitrogens of nucleotides and nucleic acids; and various nucleophilic groups in free amino acids, polypeptides, vitamins, cofactors, lipids, and steroids (15). Chlorambucil specifically may react with cell surfaces and produce a concentration-dependent lysis of erythrocytes (16). However, the majority of investigators indicate that the most likely primary effect of alkylating agents is on DNA, particularly at the 7-position of the guanine moiety (17).

Previous work has shown that cysteine is not only capable of protecting against the toxic and lethal effects of radiation, but it also decreases the toxicity of certain alkylating agents by perhaps providing a high concentration of nucleophilic groups with which the alkylating agent may interact directly. Since thiosulfate does not protect against alkylating agents and is incapable of penetrating into cells and since cysteine particularly when dissolved in water rapidly reaches a high intracellular concentration, it is likely that cysteine protection occurs within the cells (15).

This, however, may not be the full explanation in the case of teratogenesis. The most effective protective dose of cysteine was not the highest dose, that is did not provide the largest amount of sulfhydryl groups, and, of greater importance, did not result in 100% effectiveness. A significant percentage of fetuses still suffered from at least one structural defect. Figure 1 shows the incidence of specific abnormalities as a function of the time of administration of chlorambucil alone and the dose of cysteine given on day 12 in combination with chlorambucil. As shown, the most susceptible period for anterior limb, tail, and kidney defects is on day 12; for brain and skull and posterior limb malformations on day 13; and for jaw and palate abnormalities on day 14. With the exception of tail defects, the degree of protection given by cysteine appears to be inversely proportional to the dose. The anterior limb and tail both appear to have equal or greater susceptibility to abnormal development in the presence of cysteine than in its absence. Moreover, the syndrome of malformation, that is the pattern of anomalies suffered by the abnormal fetuses, with cysteine administration seems to be unlike the patterns induced by chlorambucil alone on day 12, 13, or 14. This tends to suggest several possi-

bilities.

First, in light of the data that indicate a modest degree of cysteine-induced fetal toxicity (Table I), the addition of chlorambucil may ameliorate the lethal effects of cysteine—modifying these to teratogenic actions that appear to affect the anterior limb and tail more than other anatomical structures. Second, since derivatives of nitrogen mustard may cause hyperemia (18) and since chlorambucil has a very short half-life, it is possible that cysteine protects against the direct alkylation of DNA, but does not affect the vasodilation and altered capillary permeability induced by the drug. As a consequence of these effects, placental or yolk sac permeability may become abnormal leading to increased or decreased penetration by maternal metabolites. This in turn may result in the differing patterns of fetal defects in the presence of cysteine.

A practical aspect to the use of radioprotective agents as a means to inhibit teratogenesis is the possibility that human conceptuses being carried by women requiring diagnostic X-irradiation during the early critical stages of development may be spared from the teratogenic effects of X-rays by pretreatment with a protective and nontoxic drug.

Summary. Pregnant rats were given a single injection of cysteine prior to exposure to chlorambucil on day 12 of gestation. This treatment caused a significant 6-fold increase in structurally normal surviving fetuses when compared to chlorambucil alone. The abnormal survivors demonstrated a less severe syndrome of anomalies that was not similar to the patterns of defects associated with chlorambucil treatment on other days of gestation.

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