Cyclophosphamide Therapy in Prepubertal Rats and Subsequent Reproductive Performance

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Berry, C. L. (1971). *Archives of Disease in Childhood*, 46, 709. **Cyclophosphamide therapy in prepubertal rats and subsequent reproductive performance.** In an experimental system using rats, prepubertal treatment with cyclophosphamide was associated with slightly reduced fertility of the adult female. No malformations were noted in the offspring, and there was no change in the size of the rat pups produced. Reduction in litter size was apparent by day 11 of gestation, suggesting that losses before or around implantation were the most likely cause of the reduction in litter size.

The relevance of these results to cytotoxic drug therapy in the nephrotic syndrome in childhood is considered.

The use of cytotoxic drugs in therapy of selected cases of the nephrotic syndrome in childhood (Moncrieff *et al.*, 1969; Barratt and Soothill, 1970), an essentially 'non-malignant' disease, raises certain problems in terms of possible long-term effects of the drug on the developing gonads. Work on the mouse by Miller and Cole (1970) has suggested that ovarian changes may occur after prolonged treatment with high doses of cyclophosphamide, and these authors report similar effects found at necropsy in a 13-year-old girl. Other work in the rabbit (Gerlinger, 1966) supports these observations.

This study is concerned with the reproductive performance of rats treated with cyclophosphamide prepubertally. Two regimens were used: a single high dose, and a dose resembling that used in therapy of the nephrotic syndrome in its effect on the circulating white cell count. After an interval (3 or 6 months) animals treated in this way were used in breeding experiments.

Materials and Method

Wistar rats, maintained as a closed colony, were used in all experiments. They were maintained under standard animal house conditions with a constant temperature environment and fed 'Oxoid' diet with water *ad libitum*. After weaning at 4 weeks, cyclophosphamide (W.B. Pharmaceuticals Ltd.) was injected as a single dose intraperitoneally in the high dose

regimen (150 mg/kg body weight). In the low dose experiment, 3 mg/kg body weight was given for five days by the same route—this dose reduced the total white cell count by approximately half (mean 9700 → mean 4600).

In both instances animals were kept for a further 3 or 6 months when breeding was begun. Treated males and females were paired with age-matched animals of the opposite sex. Age-matched untreated pairings were used as controls. Records of the time that pairs were together, resorption rates in animals sacrificed on day 11, and crown-rump lengths and weights of pups at term were noted. Approximately half the pups were examined for abnormalities by a modification of Wilson's free-hand sectioning technique, and the remainder by alizarin staining.

Results

Normal litter size in our colony is 9.3 (SE 0.3), crown-rump length 4.25 cm (SE 0.02), and weight 5.42 g (SE 0.04). Though weights at term have been recorded in these experiments, the difficulty of the assessment of the effects of feeding was felt to make this measurement unhelpful in terms of showing minor differences. Normal resorption rate in our colony at 11 days, based on the examination of 80 litters, is 0.75 resorptions/litter when the litter size is 10.82 ± 0.01 (SE), giving a rate of 7%.

Results from animals treated with the high dose of cyclophosphamide at 4 weeks of age are shown in Table I. Treated rats (aged 6 months) were paired with normal animals of the same age.

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TABLE I
Cyclophosphamide (150 mg/kg body weight). Results of Breeding from Treated Males and Females 6 Months After Administering Drug

Pairing	Treated Females/ Normal Males	Treated Males/ Normal Females
Pair weeks/litter*	69 (11)	29 (17)
No. of litters	7	6
Weight mean (g)	5.35	5.58
CR length (cm)	4.25	4.24
(SE)	(0.04)	(0.04)
Litter size	7.59	9.6
Mean (SE)	(1.07)	(0.77)

*The derivation of 'pair weeks/litter' is explained in the text. The figures in parentheses in this row refer to the time taken for age-matched control pairs to produce a litter. Elsewhere, figures in parentheses refer to the standard error of the mean.

Breeding animals were used in various combinations. The number of pairs was multiplied by the time in weeks they were together, and this figure divided by the number of litters produced to give pair weeks/numbers of litters—an index of infertility. The figures in parentheses are the results for age-matched untreated controls. There is an obvious increase in the time necessary for production of a litter in test animals. Crown-rump lengths and litter sizes are shown with their standard errors. Examination of pups from these litters showed no evidence of visceral or skeletal abnormality.

The prolonged time taken for age-matched control animals to produce litters in this experiment led to a reduction in the interval between exposure to the drug and breeding in the 'low dose' regimen. Here animals were treated at 4 weeks of age and breeding started at 4 months—in this way it was hoped to avoid age-dependent loss of fertility. Results from these animals are shown in Table II. Resorption rates were determined on 6 animals from each pairing. No abnormalities were found by free-hand sectioning or alizarin staining in animals delivered spontaneously.

Discussion

There are many possible objections to studies of this kind. Comparative data are difficult to assess and attempts to calculate 'life span fractions' in man and rat are not likely to produce biologically equivalent periods. However, such comparative studies may give more useful information at a cellular level, and the effect of cyclophosphamide on the prepubertal gonad, with assessment of later reproductive capacity, may provide evidence as to the likely effects of the drug in man.

Very large doses of the drug reduced fertility and litter size in treated females, but the fetuses produced were normal in size and form. Treated males showed a less marked effect on fertility and no effect on litter size. The numbers of litters produced do not permit an adequate statistical treatment of the reduction in litter size.

In the low dose experiment there was no effect on fertility as assessed by the time taken for a 'pair' to produce a litter. The reduction in litter size in treated females is apparent; this effect is shown to differ significantly from normal (determination of the standard error of the difference between the two means). The effect is present by day 11 of gestation, at which time resorption rates are normal, suggesting that preimplantation or 'failed implantation' losses account for the difference.

Miller and Cole (1970) have pointed out that in addition to structural changes in the ovary produced by the drug there is a cyclophosphamide effect on the ovum in mice, the mechanism of which is not clear. It is possible that this compound produces changes that reduce fertility by the production of abnormal zygotes—such changes result in fewer implantations in treated animals. The effects of cyclophosphamide on an apparently inert cell may be due to its effects on DNA repair mechanisms important in early divisions, an effect that would be manifest after fertilization (see Alexander, 1969). Epstein *et al.* (1970) have discussed the use of preimplantation losses in assessing the effects of possible mutagens on zygote development, and have noticed retardation, anisocytosis, and multinucleate blastomers when sperm from male mice treated with triethylenephosphoramide some weeks previously was used to fertilize normal mouse ova,

TABLE II
Cyclophosphamide (3 mg/kg Body Weight): Results of Breeding from Treated Males and Females 3 Months after Administering Drug

Pairing	Treated Females/ Normal Males	Treated Males/ Normal Females
Pair weeks/litter*	4 (4)	3 (4)
Number of pregnancies	27	19
Weight mean (g) (term)	5.72	5.30
CR length (cm)	4.26	4.24
Mean (SE) (term)	(0.03)	(0.06)
Litter size	7.9	9.7
Mean (SE)	(0.44)	(0.89)
Resorption rate (11 days)	6.6%	6.4%
Litter size (11 days)	8.56	10.67
	(0.88)	(0.03)

*The derivation of 'pairs weeks/litter' is explained in the text. The figures in parentheses in this column refer to the time taken for age-matched control pairs to produce a litter. Elsewhere, figures in parentheses refer to the standard error of the mean.

together with reduction in the fertilization rate. Thus in both male and female animals more sophisticated methods than those used here may show effects of cytotoxic drugs on the prepubertal gonad.

It appears from these experiments that successful implantation is followed by normal pregnancy and the delivery of a normal rat pup. This suggests that female children treated with this drug in childhood may have a slightly reduced fertility but are not likely to show a higher than normal incidence of malformation in their offspring. In this connexion it is of interest to note that Alexander (1969) and Di Paolo (1969) have pointed out that cyclophosphamide and other alkylating agents are less teratogenic than might be expected in man.

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