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A COMPARISON OF THE ANTIFERTILITY EFFECTS OF ALKYLATING AGENTS AND VINCA ALKALOIDS IN MALE RATS

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1 The anti-fertility effects of cyclophosphamide, nitrogen mustard, vincristine and vinblastine were studied and compared in male rats.

2 The effects of the drugs on body weight and haematological values were used to monitor the pharmacological actions of the drugs.

3 All four drugs impaired fertility, the severity of the impairment depending on dose and duration of treatment.

4 Testicular size and histological appearances remained mostly normal, even in infertile animals, but seminiferous tubules were fewer in number and maturation arrest at the spermatid level was evident in some sections.

5 Recovery of drug-induced infertility occurred in 64% of treated animals, 9 to 40 weeks after cessation of treatment.

6 Morbidity and mortality were much higher with alkylating agents than with vinca alkaloids for approximately similar degrees of impairment in fertility.

Introduction

Many alkylating agents have been found to produce infertility, both in man and in animals (Jackson, Fox & Craig, 1959; Miller, 1971; Fairley, Barrie & Johnson, 1972). However, virtually no information is available about the effect of vinca alkaloids on fertility, although these drugs are among the most widely used cytotoxic agents. Clinical data supplied by the manufacturer have been based on unpublished single case reports, (information supplied by Dr H.O. Wooller, Medical Director, Lilly Industries Pty. Ltd., Sydney, Australia) and studies with rats have been chiefly concerned with morphological changes in testicular tissue, without *in vivo* assessment of fertility (Welter, 1963; Bustos-Obregon & Feito, 1974). In the present study, the alkylating agents cyclophosphamide (Cp) and nitrogen mustard (NM), and the vinca alkaloids, vincristine (VCR) and vinblastine (VLB), have been administered to male rats and their effect on fertility determined.

Preliminary experiments established a range and frequency of drug doses that were associated with measurable pharmacological effects in the animals. This dosage range encompassed, but also exceeded, the doses used in clinical medicine.

Methods

Albino Wistar rats were kept in a controlled environment at a temperature of 21°C. Standard rat pellets (Superstock Company, Queensland) and water were available to the animals *ad libitum*. All rats used were at least 12 weeks old: females weighed 130 to 250 g and males 230 to 350 g. All rats were tagged and weighed on receipt. The weights were arranged in ascending order, and allocation to treatment or control groups was made by use of random numbers.

Cp (Endoxan Asta, Mead Johnson, Australia) in aqueous solution was administered by intragastric infusion. NM (The Boots Company, Australia Pty. Ltd.) as well as VCR and VLB (Lilly Industries Pty. Ltd., Sydney, Australia) were dissolved in 0.9% w/v NaCl solution (saline) and administered by the tail vein. The drug regimens used are indicated in Table 1. In each case, appropriate control animals received the drug solvent as a placebo under identical conditions. Concentrations of the four drugs were adjusted in such a way that all animals received 2 ml/kg body weight of the solution.

The effects of administered drugs were assessed by the following parameters: (a) mortality rate; (b) change in body weight by weekly weighing; (c) hae-

matological values; 5 or 6 animals were randomly chosen from each group and bled from their tail veins. Estimations of haemoglobin concentration were made and total white cell count was performed with a Coulter counter (model 'S'). These measurements were made 4 to 6 days after each drug administration; and (d) assessment of fertility. Each treated or control male rat was caged with three mature females for 7 days. The ovarian cycle of females was monitored by serial smears of vaginal epithelium. All females were killed 9 to 11 days after removal of the male from the cage. This procedure yielded three measures of reproductive function. Failure to impregnate any of the 3 females was regarded as evidence of sterility of the male. An increase in the percentage of unproductive matings and/or a decrease in the number of foetal implants per pregnancy were evidence of subnormal fertility ('sub-fertility'); (e) structural testicular damage was evaluated by testicular weight and histology. After mating studies, all fertile animals were killed and in addition, a sample (1 to 2 animals) of infertile animals was killed in order to examine testicular histology. The testes were removed and weighed. They were preserved in Bouin's solution and stained with haematoxylin and eosin. Those males which proved infertile in mating experiments and were not killed were kept to assess subsequent recovery of fertility by serial mating experiments.

Results

Because of the large numbers of animals involved, the experiments with the two types of drugs were undertaken sequentially, but under identical conditions. Concurrent controls were used in each experiment but the cumulative data of 50 control animals showed no significant variation and were therefore pooled.

Mortality

The only deaths to occur resulted from the high dosage regimens of Cp and NM. A total Cp dose of 200 mg/kg caused 10% deaths when given at a rate 20 mg/kg per week, but the death rate reached 44% when the rate of administration was 50 mg/kg per week, for 4 weeks. Thirty-one percent of animals died during high dose treatment with NM.

Body weight

All drugs retarded the growth rate of the animals, but only those receiving high dose Cp actually lost weight during treatment. Normal growth resumed rapidly after cessation of the drugs.

Haematological values

In control animals, the haemoglobin concentration was 15.9 ± 1.5 g/dl, and the white cell count $13.5 \pm 2.6 \times 10^9$/l. Medium and high dose treatment with Cp and NM caused statistically significant leukopenia ($P < 0.01$), the lowest white cell count being $5.0 \pm 1.4 \times 10^9$/l with high dose NM. Anaemia was only of moderate degree, even with the highest dose of all drugs, the lowest haemoglobin value being 10.6 ± 3.7 g/dl with the highest dose of Cp. The vinca

Table 1 Drug treatment for comparison of antifertility effects of alkylating apents and vinca alkaloids in male rats

Drug	*Interval between doses*	*Dose level (per kg weight)*	*No. of doses*	*Total drug administered (per kg weight)*
Cp	1 week	10 mg	5	50 mg
		10 mg	10	100 mg
		20 mg	5	100 mg
		20 mg	10	200 mg
		50 mg	4	200 mg
NM	2 weeks	100 μg	12	1.2 mg
		250 μg	12	3.0 mg
		500 μg	12	6.0 mg
VCR	1 week	40 μg	9	0.36 mg
		60 μg	9	0.54 mg
		100 μg	9	0.9 mg
VLB	1 week	80 μg	9	0.72 mg
		250 μg	9	2.25 mg
		400 μg	9	3.6 mg

Cp = cyclophosphamide, NM = nitrogen mustard, VCR = vincristine, VLB = vinblastine.

alkaloids caused moderate anaemia and no significant leukopenia.

Assessment of fertility

The effect of the cytotoxic drugs on the three measures of fertility outlined in the Methods section is shown in Table 2. A dose-dependent effect was observed with all drugs. Cp was the only drug to produce significant reductions in the number of foetal implants, and also caused the greatest effect on the rate of impregnation. Males which proved totally infertile were only observed after the highest dose of therapy with all drugs except VCR, where this effect was also noted at the medium dose. Overall, at the dosages employed, Cp proved most damaging to fertility and NM was least so; VCR and VLB had intermediate effects.

Table 2 Effects of different doses of four cytotoxic drugs on fertility of male rats

	Treatment group			*Assessment of fertility*		
Drug	*Dose (per kg)*	*No. of doses*	*No. of animals*	*Infertile animals (% of group)*	*Failed impregnations (% of)*	*No. of foetal implants (mean ± s.d.)*
	Control		50	2	21	9.5 ± 2.3
Cp	10 mg	5	22	9	37	7.6 ± 2.3*
	10 mg	10	20	10	42**	6.8 ± 2.5**
	20 mg	5	22	10	53**	4.7 ± 2.3**
	20 mg	10	18	17	39*	5.8 ± 3.0**
	50 mg	4	26	65**	83**	4.3 ± 2.8**
NM	100 μg	12	16	0	21	9.0 ± 2.2
	250 μg	12	16	13	31	8.3 ± 2.4
	500 μg	12	17	24*	41*	8.1 ± 2.2
VCR	40 μg	9	9	0	27	8.0 ± 1.6
	60 μg	9	12	33*	53**	8.5 ± 2.0
	100 μg	9	11	45**	54**	8.5 ± 2.4
VLB	80 μg	9	9	0	33	7.9 ± 2.6
	150 μg	9	22	18	46*	8.6 ± 2.0
	400 μg	9	16	56**	58**	8.7 ± 2.2

Cp = cyclophosphamide, NM = nitrogen mustard, VCR = vincristine, VLB = vinblastine.
Result significantly different from control: *P = 0.05; **P = 0.01. Chi square test used for fertility, t test used for foetal implants

Table 3 Recovery from drug-induced infertility

Treatment group			*Time after cessation of therapy (weeks)*		
Drug	*Dose (per kg weight)*	*No. of animals*	9–14	15–28	29–40
Cp	10 mg	2	2F	—	—
	20 mg	3	3F	—	—
	50 mg	11	2F 9I	6F 3I	—
NM	250 μg	2	2I	2I	2I
	500 μg	4	4I	4I	1F 3I
VCR	60 μg	3	1F 2I	1F 1I	1I
	100 μg	2	2I	1F	1F
VLB	250 μg	2	1F 1I	1I	1I
	400 μg	4	1F 3I	1F 2I	2I

Cp = cyclophosphamide, NM = nitrogen mustard, VCR = vincristine, VLB = vinblastine. F indicates animal became fertile, I indicates animal remained infertile.

Testicular weight and histology

Results with a large number of normal animals (70 *in toto*) revealed that testicular weight was positively and significantly correlated with body weight ($r^2 = 0.29$, $P = 0.05$). Hence, any evaluation of the effect of cytotoxic therapy on testicular weight required reference to control animals of similar body weight, at the time they were killed. When this factor was allowed for, only high dose (50 mg/kg) treatment with Cp resulted in testicular atrophy in those animals which became infertile. Their testicular weight was 1.67 ± 0.80 g, and that of body weight matched controls was 2.25 ± 0.39 g ($t = 2.72$, $P = 0.05$). Histological examination of testicular tissue, including that of infertile animals with small testes, revealed little abnormality. Seminiferous tubules were fewer in number than in controls, and maturation arrest at the spermatid level was evident in some sections of testicular tissue from high dose Cp-treated animals. No morphological changes were seen in testes from animals treated with VCR and VLB.

Studies of recovery in fertility

Recovery of drug-induced infertility was monitored in a total of 33 rats of which 21 regained fertility 9 to 40 weeks after cessation of drug-treatment and the remaining 12 animals were killed still infertile. Table 3 indicates that the rate of recovery was high after Cp (81%) and VCR (80%) therapy, and lower after NM (17%) and VLB (50%) therapy. The highest dose of the drugs tended to be associated with a lower rate of recovery than the medium and low doses. The small number of animals in most treatment groups precluded statistical evaluation of the differences.

Discussion

Cp and NM are polyfunctional alkylating agents with potent cytotoxic activity for both normal and neoplastic cells. The precise mechanism of action which results in cell death has not been established. It is probable that cross linking of deoxyribonucleic acid, inhibition of protein synthesis and arrest of the cell cycle in the G_2 stage are important factors which interfere with viability (Wheeler, 1967). Tissues which contain a high proportion of dividing cells, such as the germinal epithelium of the testis, are particularly susceptible to damage (Sternberg, Philips & Scholler, 1958; Karnofsky, 1967). The vinca alkaloids are also known to arrest the cell cycle at the stage of metaphase and to suppress nucleic acid synthesis (Creasy & Markiw, 1964). It is therefore not surprising that VCR and VLB share some of the biological effects of alkylating agents.

The antifertility effect of the drugs evaluated was found to depend on the nature of the drug, on the dosage and on the duration of therapy, although the latter aspect was only measured for Cp. At the lowest dose of this drug, a reduction in the number of foetal implants was the only evidence of subfertility in the animals tested. This effect of Cp has been described previously with low dose, short term administration of the drug (Botta, Hawkins & Weikel, 1974). Further impairment of fertility, manifested by an increased number of unproductive matings, could be achieved by either increasing the dose of Cp or prolonging the administration of the drug at the same dose. A large increase in dosage resulted in sterility in over half the animals tested. When NM was used, only the highest dose resulted in significant infertility and even at this dose level, no reduction in the number of foetal implants was evident. The degree of impairment of fertility was approximately similar for VCR and VLB. However, when a comparison is made between the effects of alkylating agents and vinca alkaloids, a substantial difference merges. The doses of Cp and NM required to produce significant infertility were associated with mortality rates of 44% and 31% respectively. These high death rates probably reflect the marked leukopenia which followed the use of high dose alkylating agents. In contrast, significant infertility was produced with both VCR and VLB in doses which did not result in any deaths and caused only moderate bone marrow suppression. In man, alkylating agents have been shown to cause infertility in doses which are unaccompanied by significant systemic toxicity. Thus, a daily dose of only 1 to 2 mg/kg of Cp caused azoospermia in all of 31 men who received this treatment (Fairley *et al.*, 1972). Testicular histology in such patients reveals marked atrophic changes, with most tubules consisting chiefly of Sertoli cells (Sherins & de Vita, 1973). The relative lack of histological changes in the testes of animals in the present study emphasizes species differences in tissue response to cytotoxic agents. Germinal epithelial hypoplasia and maturation arrest in rat testes have indeed been produced both with NM (Goldeck & Hagenah, 1951) and VLB (Bustos-Obregon & Feito, 1974) but only with single, large doses of these drugs, which would be uniformly lethal if given repetitively.

The pattern of recovery in drug-induced infertility has been approximately similar in men treated with Cp alone (Buchanan, Fairley & Barrie, 1975) and in men treated for lymphomas with combinations of alkylating agents and vinca alkaloids (Sherins & de Vita, 1973; Roeser, Stocks & Smith, 1978). For this and other reasons, the assumption had been made that VCR and VLB in conventional doses had little effect on gonadal function. The present study suggests that this is not so, and that the potential effects of vinca alkaloids on reproductive function must

become a consideration in their use. Such a consideration is particularly relevant in view of the increasing use of VCR as an immunosuppressive agent in the treatment of non-neoplastic diseases with a prolonged life expectancy.

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