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Effects of vincristine on developing hamster embryos

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Using dosages and a route of administration which resembled those used clinically in humans, the effects of vincristine on developing hamster embryos were evaluated. The results showed that the drug exerted a highly toxic effect in a dose-dependent manner; however, it exhibited only a sporadic teratogenic (gross malformation) effect. The data on DNA synthesis indicated involvement of internal organs and structures, which needs to be verified. Overall, the teratogenicity of the drug was more pronounced during pre-organogenesis than during the organogenesis period. It was suggested that, in contrast to the data reported in the literature, the different biological response to vincristine in hamster may relate to the use of the dose–route combination, and is potentially relevant to developmental and transplacental carcinogenesis studies.

Key words: Hamster, teratogenicity, vincristine.

Introduction

Vincristine (VCR), a vinca alkaloid, is used extensively in anticancer therapy. The drug is believed to exert its biological effect by arresting the mitotic cells in metaphase.[1,2] The drug can also be potentially used in transplacental carcinogenesis work in animals, such as the hamster, because, unlike other rodents, VCR does not induce chromosomal abberation in hamster fibroblasts;[2] hence, the mode of drug administration and its effect on the developing organism are also of significance. Studies reported during the past 30 years suggest that VCR, when administered to pregnant rodents and primates during embryogenesis, exerts a variable toxic or teratological effect (reflected by the presence of gross malformation) in the fetuses.[3–10] A critical analysis of these studies shows that the observed variability in the biological response to the drug may be related to differences in the route of drug administration, time of drug injection and dosage used. In addition, none of these studies evaluated the drug response either throughout the entire period of organogenesis or by the dose–route combination proposed for humans.[11] In this preliminary report we describe the effects of VCR on the developing hamster embryo throughout the period of active organogenesis, i.e. at a time when cells are proliferating rapidly in the embryo. It has been repeatedly suggested that in order to derive a meaningful extrapolation of data to humans, teratological testing of a drug in animals should be undertaken by using both the dose and route of administration similar to that used in humans. Unlike previously reported VCR teratological studies,[3–10] in which the drug was injected by either the intraperitoneal or intramuscular routes, we have injected the drug intravenously, which is the preferred clinical mode of drug adminisration in humans[11] and which has been hitherto untested in animals. It is hoped that the proposed approach will be potentially useful in developmental as well as transplacental carcinogenesis work.

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Materials and methods

Environmental acclimatization and breeding of Golden Syrian hamsters (90±5 g) have been described previously.[12] A single intravenous injection of VCR (Oncovin, Eli Lilly, Toronto, Canada) dissolved in 0.5 ml saline was given to hamsters on different days of gestation, i.e. encompassing a period from the post-implantation stage through organogenesis (Table 1). Controls received 0.5 ml saline. At each time of VCR treatment, the doses were adjusted until the resorption rate reached 90%. Animals were killed on day 15 of gestation and resorption sites were counted. The fetuses were recovered, weighed and examined for gross malformations. The data were evaluated by ANOVA.

In a separate experiment, three control and three VCR-treated (0.5 mg/kg) embryos were obtained 3 h after the injection on appropriate days (Table 2) and incubated in 2 ml Dulbecco's modified Eagle medium containing 10% calf's serum and 0.03 ml of [^{3}H]thymidine ([^{3}H]Tdr; ICN, Montreal, 2.0 Ci/mmol) for 3 h at 70°C in an environment of 5%

Table 1. Effects of a single intravenous injection of vincristine at different times during prenatal development in hamster

Day of injection	Dose (mg/kg)	No. of litters	No. of live fetuses	No. of resorption (%)	No. of malformed fetuses (%)	Mean fetal weight (g) (mean ± SD)
5	control	3	26	3 (10)	0	2.02 ± 0.20
	0.25	3	34	2 (6)	3 (9)	1.76 ± 0.29
	0.5	5	39	13 (25)	16 (41)	1.81 ± 0.37
	0.75	3	0	33 (100)	0	—
6	control	3	34	1 (3)	0	2.2 ± 0.31
	0.5	3	30	1 (3)	0	2.0 ± 0.11
	0.75	3	27	2 (7)	2 (7)	2.2 ± 0.20
	1.0	3	0	26 (100)	0	—
7	control	3	35	0	0	1.99 ± 0.30
	0.5	3	38	1 (3)	0	1.99 ± 0.33
	0.75	3	25	8 (24)	1 (4)	1.95 ± 0.16
	1.0	4	1	54 (98)	1 (100)	1.75
8	control	3	32	1 (3)	0	2.04 ± 0.34
	0.25	5	50	8 (14)	1 (2)	1.81 ± 0.33
	0.5	4	46	3 (6)	0	1.77 ± 0.23
	0.75	3	20	24 (55)	0	1.54 ± 0.25
	1.0	4	26	15 (37)	0	1.58 ± 0.24
	1.25	4	22	21 (49)	0	1.47 ± 0.19[a]
	1.5	3	3	35 (92)	0	1.13 ± 0.12[a]
9	control	3	41	1 (2)	0	1.89 ± 0.22
	0.5	4	37	7 (16)	0	1.84 ± 0.35
	0.75	3	7	27 (79)	1 (14)	1.26 ± 0.32[a]
	1.0	3	1	38 (97)	0	1.5
10	control	3	37	0	0	2.02 ± 0.44
	0.25	3	34	1 (3)	0	1.85 ± 0.22
	0.5	3	31	1 (3)	2 (6)	1.74 ± 0.25
	0.75	4	12	40 (77)	0	1.69 ± 0.22
	1.0	2	0	24 (100)	0	—
11	control	3	30	2 (6)	0	1.92 ± 0.25
	0.25	3	33	0	3 (9)	2.0 ± 0.29
	0.5	3	31	0	0	2.0 ± 0.22
	0.75	3	33	2 (6)	0	1.68 ± 0.34
	1.0	4	17	31 (65)	4 (25)	1.64 ± 0.15
	1.25	2	0	23 (100)	0	—

[a] $p < 0.01$.

Table 2. Incorporation of [^{3}H]Tdr in embryo following VCR treatment of pregnant hamster.

Day of injection		d.p.m./mg protein ($\times 10^2$) (mean ± SD)
5	C	24 ± 8
	T	8 ± 3
6	C	464 ± 51
	T	163 ± 49
7	C	1683 ± 169
	T	801 ± 113
8	C	3063 ± 198
	T	2193 ± 260
9	C	8700 ± 312
	T	6657 ± 739
10	C	11150 ± 1005
	T	8396 ± 1090
11	C	24272 ± 1810
	T	19669 ± 1912

C, control; T, treated.

CO_2 and 95% air. The embryos were then processed for liquid scintillation counting as described earlier.[12] Each experiment was repeated three times. The data were analyzed by Student's *t*-test (significance level 5%).

Results

Following a single intravenous injection of VCR to hamsters at different times during gestation, 438/1035 (42%) implantation sites were resorbed (Table 1). Although the frequency of resorption increased with the dose of VCR on various days of treatment, differing amounts of drug were required during pre-organogenesis (days 5–7) and organogenesis (days 8–11) periods to induce embryotoxic responses.

On the basis of gross observations, overall, the drug showed a mild teratological response. Following VCR administration, only 35/597 (5.8%) of live fetuses showed malformations. The majority of these malformations (23/35; 65.7%) were seen following drug administration during the pre-organogenesis period. The malformation rate was higher following drug treatment on day 5 of gestation (19/73; 26%). Following VCR treatment on the remaining days, i.e. days 6–11 of gestation, the overall malformation rates varied between 0.6 and 6.1%; during this period the malformed fetuses were observed sporadically and a dose–response curve was absent. The types of malformation observed included microphthalmia, gut herniation, cleft palate and micrognathia.

The results of [^{3}H]Tdr incorporation (Table 2) show that, in comparison to controls, VCR-treated embryos showed a 53–65% reduction in DNA synthesis during pre-organogenesis (days 5–7) and a 20–30% reduction during the organogenesis (days 8–11) period ($p < 0.05$).

Discussion

The dose and route of drug administration used in the present study resembled those preferred clinically in humans.[11] The observations of the present study indicates that VCR, when injected in pregnant animals, is embryotoxic. This observation is consistent with the data reported in earlier studies.[3,6,8–10] However, with the exception of intravenous injection of VCR on day 5, only a mild teratological response was observed in hamster embryos. This observation is in contrast to a dose-dependent, high malformation rate observed in other rodents following intraperitoneal administration of VCR.[6,9,10] A high malformation rate, albeit not in a dose-dependent manner, was also observed by others following intramuscular and intraperitoneal injections.[4,5,8] Clearly, differences in both species and route of drug administrations are critical in determining the teratogenicity of VCR in laboratory animals. Further, it may be noted that, unlike in mice,[9,13] VCR does not induce chromosomal abberation in hamster fibroblast cells,[2,14] which may further account for its mild teratogenic effects in hamsters. Also, unlike previous studies,[3–6,8,10] in which drug teratogenicity was observed only on a specific day during organogenesis, results of the present study show that in hamster VCR is teratogenically more potent during pre-organogenesis (days 5–7) than during the organogenesis (days 8–11) period. A similar effect of VCR during the pre-organogenesis period in mice was also observed by Sieber *et al.*[9]

In contrast to the previous studies, in which VCR was shown to cause reduction in the mean fetal weight,[6,8–10] the results of the present study show that, with the exception of highly embryotoxic doses of VCR on days 8 and 9 (Table 1), the drug does not induce any significant retardation in the fetal weight. It is possible that this difference may have resulted from differences in the route of drug administration and drug metabolism in the fetal and maternal tissues between different species of animals. However, there was a significant reduction in [^{3}H]Tdr incorporation in embryos, indicating growth retardation in the absence of, except on day 5, observable gross malformations. This may be indicative of malformation of internal organs and structures which needs to be verified.

In brief, the data of this study suggests that, unlike in other rodents, VCR administration by the preferred clinical route and dose exerts a very highly toxic but only a mildly teratogenic (gross malformation) effect on hamster embryos. DNA synthesis data, however, suggests hitherto unrecognized involvement of internal organs/structures, which needs to be verified.

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