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An Investigation of the Mouse as a Model for Vincristine Toxicity

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Summary. *The purpose of this study was to investigate the toxicologic responses of mice to vincristine (VCR), an established antitumor drug, and to compare them with those reported for dogs, monkeys, and humans. This comparison was expected to facilitate the continuing appraisal of the mouse as a model for toxicologic responses to antitumor drugs in human patients. In duplicate experiments, male B6D2F$_1$ mice were treated with 1.0, 1.5, 2.0, and 3.0 mg/kg of VCR in single IP doses. These sublethal doses corresponded to 0.25, 0.40, 0.50, and 0.80 LD$_{50}$. On posttreatment days 1, 3, 6, 10, 14, and 21, groups of mice were killed and blood and other tissues were collected for hematologic (8 tests), clinical chemical (15 tests), and histopathologic (11 tissues) evaluations. VCR produced dose-dependent body weight loss, reticulocytopenia, granulocytopenia, elevated plasma alkaline phosphatase, GPT, and GOT activities, and damage to the gastrointestinal epithelium. These reversible changes were most severe during the first 3 days posttreatment. The mouse was comparable to the dog and the monkey in reflecting the target organ toxicity of VCR in humans. Studies with additional antitumor drugs will be required before the overall predictive reliability of this model can be expressed quantitatively.*

Introduction

From the perspective of general toxicology, preclinical toxicologic evaluation of new antitumor drugs presents some unique problems and features. These drugs are cytotoxic. Otherwise, they would have no therapeutic potential in the conventional sense. Rather than dealing with a relatively safe drug and trying to elicit, predict, or extrapolate toxicity, the toxicologist is confronted with a situation in which dose-limiting toxicity is virtually guaranteed in dose ranges of therapeutic interest. The problem is to predict a safe dose for initial clinical trials. This problem is complicated by the fact that the exposed human population will be a tumor-bearing population instead of a relatively healthy one. Studies in healthy laboratory animals may not accurately reflect the responses of a compromised host to the drug. Preclinical toxicity studies of antitumor drugs are carried out in one rodent and one nonrodent species [2], but the protocols require fewer animals and shorter exposure or treatment durations than are common for other routine toxicity tests. However, these protocols have evolved as a reflection of the intended initial clinical use of a new antitumor drug, namely, abbreviated treatment of terminally ill cancer patients. Although the use of the beagle dog as the nonrodent species permits comparison with extensive data on normal and abnormal physiologic responses of beagles, use of the mouse as the rodent species permits less reliance on historical toxicity data than might be possible with other rodent species, particularly the rat. Quantitative dose-response comparisons between mouse and man are quite reliable [7], however, and the greatest concern about using the mouse as a model for antitumor drug toxicity has to do with the ability of this species to provide qualitative, target-organ predictions that are reliable for man [3]. The present report provides information relevant to this concern on an additional drug [9], vincristine.

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Materials and Methods

Vincristine (VCR) was supplied by the Developmental Therapeutics Program, Division of Cancer Treatment, NCI (Bethesda, MD, USA). It was dissolved in aqueous sodium chloride (0.9 g NaCl/100 ml) and diluted so that 0.1 ml/10 g body weight provided the dosages. VCR was administered in single IP doses.

Young, adult male C57BL/6 × DBA/2F$_1$ (i.e., B6D2F$_1$) mice supplied by Simonsen Laboratories, Gilroy, CA, USA were used. Each mouse weighed 24–27 g at the time of treatment, and the mice were treated on the basis of their individual body weights. Husbandry in our laboratories has already been described [10]. The mice were caged individually and were fed Wayne Lab-Blox™ F6 (Allied Mills, Inc., Chicago, IL, USA) and tap water ad libitum. A total of 700 mice was used.

Two experiments using 350 mice each were conducted. In each experiment the mice were randomly divided into groups of 70, and each group received the following VCR doses: 0 (diluent control), 1.0, 1.5, 2.0, or 3.0 mg/kg. These VCR doses approximated to 0.25, 0.40, 0.50, and 0.80 LD$_{50}$ (LD$_{50}$ = 3.85 mg/kg, ref. 9). On days 1, 3, 6, 10, 14, and 21 (day of treatment = day 0), 10 mice from each group were killed. Procedures for euthanasia, blood collection, sample preparation, necropsy, histotechnology, hematology, and clinical chemistry have been described elsewhere [10, 11]. In five individual blood samples, the erythrocyte, leukocyte, differential leukocyte, platelet, and reticulocyte counts, and hemoglobin and packed cell

volumes were determined. The marrow cell count was determined from a sample of femoral marrow. Individual plasma samples were prepared from the remaining five mice for the determination of glucose, urea nitrogen, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, alanine transaminase (GPT), aspartate transaminase (GOT), uric acid, inorganic phosphorus, calcium, potassium, sodium, and chloride.

Body weights were determined on the day of treatment, on all sacrifice days, and on day 30. Ten mice in each group were predesignated as lethality controls and were observed daily for signs of toxicity and death. The survivors were euthanized on day 30.

Results

The single-dose LD_{10} value for VCR [9] in male $B6D2F_1$ mice has been determined to be 3.5 mg/kg. Lethality in the duplicate experiments described here was two of 40 and three of 40 for mice that received 3.0 mg/kg of VCR and were observed for 10–30 days. I shall therefore refer to all the dosages used in these experiments as sublethal. The principal signs of toxicity were body weight loss, reticulocytopenia, and elevated plasma enzyme activity. Less striking changes were observed in granulocyte counts in both experiments. Thrombocytopenia occurred after 3.0 mg/kg of VCR in one experiment only.

Body weight changes are presented in Table 1. Weight losses were related to VCR dosage. Weights reached a nadir on day 3 and exhibited a trend toward recovery during the subsequent week. The severity of reticulocytopenia and the time required for recovery were also dose-related. Reticulocyte counts reached a nadir (Table 2) on day 3 and returned to control values between days 6 and 10. Granulocytes seemed less sensitive to VCR. Granulocyte counts (Table 3) were reduced to the lower limit of the reference range and below only by the highest dose of VCR (3.0 mg/kg). This nadir also occurred on day 3. Table 4 presents data on three plasma enzymes that were elevated on day 1 (24 h posttreatment) but that exhibited activities within the reference ranges by day 3. The magnitude of increased activity on day 1 was dose-related. Although the transaminases, particularly GPT, are usually predictive for some degree of hepatotoxicity, no hepatic lesions were observed histopathologically. Alkaline phosphatase is less tissue-specific, and the changes in plasma alkaline phosphatase activity may have reflected effects of VCR on the gastrointestinal epithelium [19], especially in the small

Table 1. Mean body weight change (%) in mice treated with vincristine

Dosage (mg/kg)	Days after treatment					
	1	3	6	10	14	21
Diluent	− 0.7	3.9	1.5	2.3	3.9	4.7
1.0	− 4.1	− 8.5	− 0.7	− 1.5	3.2	7.9
1.5	− 6.6	− 12.0	− 2.3	− 1.9	1.6	2.3
2.0	− 7.7	− 16.2	− 5.4	− 0.8	2.3	7.4
3.0	− 10.6	− 19.8	− 17.2	− 3.1	0.7	4.3

Table 2. Reticulocytopenia in mice treated with vincristine

Dosage (mg/kg)	Days after treatment[a]			
	1	3	6	10
Diluent	7.9 ± 1.0[b]	11.6 ± 6.0	10.3 ± 8.5	11.9 ± 6.8
1.0	9.0 ± 3.4	1.2 ± 1.4	9.4 ± 9.2	13.2 ± 4.2
1.5	4.7 ± 0.8	0.8 ± 0.5	10.9 ± 5.9	14.4 ± 6.8
2.0	7.0 ± 2.3	0	11.4 ± 4.8	12.0 ± 4.6
3.0	4.6 ± 1.7	0	2.3 ± 2.6	11.6 ± 5.8

[a] Data for subsequent days were not remarkably different and are not shown

[b] Mean ± SD × 10^{-4}/mm^3 for reticulocyte counts of five mice from a single experiment. Our revised reference range for reticulocyte counts in male $B6D2F_1$ mice [8] is 2.8×10^4 to 33.4×10^4/mm^3 ($n = 732$)

Table 3. Granulocyte counts in mice treated with vincristine

Dosage (mg/kg)	Days after treatment[a]		
	1	3	6
Diluent	1.8 ± 0.7[b]	1.9 ± 0.5	2.4 ± 1.2
1.0	1.6 ± 0.7	1.6 ± 0.8	1.9 ± 1.1
1.5	1.9 ± 1.0	1.5 ± 1.0	2.1 ± 1.3
2.0	3.2 ± 1.6	1.0 ± 0.7	1.7 ± 0.5
3.0	2.6 ± 0.5	0.7 ± 0.5	1.8 ± 0.3

[a] Data for subsequent days were not remarkably different and are not shown

[b] Mean ± SD × 10^{-3}/mm^3 for five mice from a single experiment. Our revised reference range for granulocyte counts in male $B6D2F_1$ mice [10] is 0.7×10^3 to 4.6×10^3/mm^3 ($n = 733$)

Table 4. Effects of vincristine on plasma enzyme activity of mice

Dosage (mg/kg)	Days after treatment[a]					
	Alkaline phosphatase (IU/l)		GPT (IU/l)		GOT (IU/l)	
	1	3	1	3	1	3
Diluent	72 ± 20[b]	65 ± 12	42 ± 9	40 ± 7	147 ± 106	199 ± 88
1.0	94 ± 23	74 ± 13	53 ± 9	30 ± 4	163 ± 66	160 ± 82
1.5	109 ± 21	79 ± 14	63 ± 10	37 ± 7	273 ± 79	172 ± 92
2.0	119 ± 15	71 ± 10	88 ± 8	39 ± 14	422 ± 73	272 ± 179
3.0	155 ± 30	77 ± 4	107 ± 24	44 ± 9	433 ± 105	209 ± 95

[a] Data for subsequent days were not remarkably different and are not shown

[b] Mean ± SD of five mice from a single experiment. Our revised reference ranges for these plasma enzyme activities in male $B6D2F_1$ mice [10] are: alkaline phosphatase, 35–96 IU/l ($n = 574$); GPT, 17–77 IU/l ($n = 573$); GOT, 54–298 IU/l ($n = 572$)

Table 5. Epithelial hypertrophy, hyperplasia, and macronucleosis in the small intestine of mice treated with vincristine

Dosage (mg/kg)	Days after treatment			
	Experiment 1		Experiment 2	
	1	3	1	3
0	0/5[a]	0/5	0/5	0/5
1.0	5/5 (1.0)	0/5	4/5 (0.8)	0/5
1.5	5/5 (1.6)	0/5	5/5 (1.0)	0/5
2.0	5/5 (2.0)	0/5	4/5 (1.2)	0/5
3.0	5/5 (2.0)	0/5	5/5 (1.6)	0/5

[a] The numerator is the number of affected mice, the denominator is the number of mice studied, and the number in parentheses is the mean degree of severity where 0 = no lesion, 1 = minimal, 2 = moderate, and 3 = severe

intestine. These lesions were qualitatively similar to those produced by other antitumor drugs described previously [8]. Moderate epithelial changes appeared within 24 h posttreatment (Table 5) and were resolved by day 3. This time course paralleled the plasma enzyme changes observed (Table 4). The mean degree of lesion severity derived from semiquantitative histopathologic evaluation was dose-related and consistent in the two experiments (Table 5).

Discussion

VCR was introduced into clinical practice about 1960. Because of its usefulness as an antitumor drug, interest in its pharmacologic and toxicologic properties has continued to the present [4, 13, 14, 17]. In spite of intensive interest and extensive research, a gap remains in available data on the qualitative and quantitative toxicity of VCR in mice. Previous studies [1, 16] have provided some information on the responses of mice to VCR, but these data have not been sufficiently extensive to permit a comparison of the responses of mice with those of humans. At a time when predictions of the clinical toxicity of antitumor drugs depended on studies in dogs and monkeys [12], a retrospective study of VCR [6] revealed a good prediction of clinical toxicity by these two species. Revision of the National Cancer Institute's preclinical toxicology protocol to include intravenous toxicity studies in mice [2] has required a continuing reassessment of the predictive reliability of the mouse as a model for antitumor drug toxicity as appropriate data continue to become available [3].

The present studies of VCR in mice conform to a carefully standardized approach reviewed recently [9] and applied to a number of antitumor drugs [8, 9]. For an impression of the usefulness of each model, one may compare these data and similar data from dogs and monkeys [1, 6] with the well-documented effects of VCR in humans [5, 15, 18]. This comparison is summarized in Table 6. For each known major toxic effect of VCR in humans, the presence or absence of the effect in the animal species is indicated. Compared with the dog and the monkey, in the present study the mouse failed to predict anemia. However, doses that produced anemia in dogs also produced distinct morbidity and death. All doses used in the present study were sublethal. Moreover, reticulocytopenia, a sensitive indicator of toxicity to the erythron, was

Table 6. Predictability of animal species for toxic effects of vincristine in man

Toxic effect in man	Toxicity predicted by[a]		
	Dog[b]	Monkey[c]	Mouse
Hematologic			
Leukopenia[d]	+	+	+
Anemia	+	+	−
Reticulocytopenia	NR	NR	+
Clinical chemical			
GOT (AST)	★	★	★
GPT (ALT)	NR	NR	★
Hepatic necrosis	−	−	−
Gastrointestinal	+	+	+
Neurologic	+	+	+[e]

[a] +, toxicity occurred in animal and man; −, toxicity occurred in man but not in the animal species; ★, sign observed in animal but not in man; NR, not reported in the reference indicated
[b] Based on report of Folk et al. [6]
[c] Based on report of Adamson et al. [1]
[d] Granulocytopenia in the mouse
[e] Based on report of Uy et al. [16]

observed in mice, suggesting that higher doses or prolonged treatment would likely have resulted in frank anemia in this species as well. Leukopenia was very mild, and the total leukocyte counts were not reduced below the reference range (i.e., leukopenia was not statistically significant; data not shown). An indication of granulocytopenia was observed, however (Table 3). Elevated blood transaminase activities, observed in all animal species, have not been reported in humans. Conversely, autopsy studies of some patients who received VCR have revealed hepatic necrosis [5], but a histopathologic correlate of transaminase elevation in animals has not been observed. The data (Table 6) suggest that the mouse is at least comparable to the dog and the monkey for predicting or reflecting the target organ toxicity of VCR. Advantages of the mouse as a model for preclinical toxicologic evaluations of antitumor drugs have been discussed previously [9]. Additional studies will be required before the predictive reliability of this model can be compared for several drugs and expressed quantitatively.

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