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The mouse as a model for predicting the myelosuppressive effects of anticancer drugs

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Summary. We evaluated 17 clinically used anticancer drugs for their effects on total WBC and absolute neutrophil counts in BDF_1 mice. These drugs were selected from three categories, based on their myelosuppressive effects in man: myelosuppression is dose-limiting; myelosuppression occurs but is not dose-limiting; or myelosuppression does not occur. The ability of each drug to cause myelosuppression in mice was determined by its effect on the neutrophil count 4 days after single-dose treatment. The neutropenic effect of the maximum tolerated dose (LD_{0-20}) of each drug was characterized as marked (>65%↓), moderate (35%–65%↓), or minimal (<35%↓) to correspond with the three clinical categories of myelosuppression. The neutropenic effects in mice correctly predicted (true + or true −) the myelosuppressive effects in man for 13 of the 17 drugs (76%). Marcellomycin and the platinum complexes cisplatin, carboplatin, and spiroplatin did not cause neutropenia at maximum tolerated doses. These represent false-negative predictions, since the drugs are myelosuppressive in man. The results with the platinum complexes indicate that this method is not suitable as a means of screening these agents for myelosuppression. Excluding the platinum complexes, the predictions were correct for 12 of 13 drugs (92%). Therefore, this model is considered a good predictor for the myelosuppressive effects of anticancer drugs in man (except platinum complexes) and can be used effectively to screen drugs for this toxicity. However, it is important that drugs identified by this system as being less myelosuppressive than the reference agent(s) be evaluated further, since all the incorrect predictions were false negatives.

Introduction

The most common dose-limiting toxicity of anticancer drugs encountered in man is myelosuppression [6, 8]. An important objective of anticancer drug discovery programs is the identification of agents which are less myelosuppressive than the ones currently available for use in clinical practice. Obviously, an efficient and predictive experimental test system is needed to screen new drugs for myelosuppressive effects. We have previously reported [3, 4] on the development of a model in mice to test drugs for effects on total white blood cell counts (WBC). We now report the results of evaluations of 17 clinically used anticancer drugs for effects on absolute neutrophil counts in BDF_1 mice to determine the ability of the mouse to predict for myelosuppression in man.

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Materials and methods

Animals. These studies were done in male BDF_1 mice weighing $25–29_1$ g, obtained from the Charles River Laboratories, Wilmington, Mass. The mice were housed in wire cages and offered free access to feed and tap water.

Drugs. The anticancer drugs tested in these studies were selected from three categories based on their myelosuppressive effects in man: (a) myelosuppression is dose-limiting; (b) myelosuppression occurs but is not dose-limiting; and (c) little or no myelosuppression occurs. The drugs were dissolved in saline or water, except for BCNU which was dissolved in absolute ethanol and diluted with saline, immediately prior to IV administration (IP for BCNU). The concentrations of drugs in solution were such that the mice received the appropriate dose in a volume 0.25 ml/mouse for each IV injection or 0.5 ml/mouse for each IP injection.

Experimental design. Mice in groups of 40–50/drug (10/dose) were bled from the retro-orbital plexus 2 days before drug treatment. The total WBC counts of individual mice were measured using a Model S Coulter Counter (Coulter Electronics, Inc., Hialea, Fla). The absolute neutrophil count for each mouse was determined by manually counting 100 cells from a Wright-stained whole blood smear and multiplying the percentage of neutrophils observed by the total WBC count. On day 0 the mice received a single dose of drug at 56%, 75%, or 100% (occasionally 133%) of its LD_{50} (previously determined in mice which were not bled). The mice were rebled 4 and 7 days after dosing and the total WBC counts determined. In most cases the post-treatment neutrophil counts of mice in selected treatment groups and the control mice were determined for the day 4 bleeding only, because preliminary tests indicated this was usually the nadir day for neutrophil counts. One drug, marcellomycin, did cause a neutrophil count nadir on day 7. The leukopenic and neutropenic effects of each drug were assessed by determining their effects on the total WBC and neutrophil counts at the approximate maximum tolerated dose (lethality in ≤20% of

the mice by day 14) and were ranked as marked (>65%↓), moderate (35%–65%↓), or minimal (<35%↓).

Results

The myelosuppressive effects of the anthracyclines tested are summarized in Table 1. Adriamycin, aclacinomycin, and carminomycin caused marked reductions in total WBC and neutrophil counts at nonlethal doses. Marcellomycin caused minimal to moderate leukopenia without neutropenia at doses up to the MTD. However, marcellomycin did cause neutropenia at 14.1 mg/kg, which is 1.33 times the LD_{50}.

The results from the evaluation of platinum complexes (Table 2) show that cisplatin, carboplatin, and spiroplatin caused minimal to moderate leukopenia but did not cause neutropenia. In contrast, iproplatin caused marked leukopenia and neutropenia.

BCNU caused reductions in both total WBC and neutrophil counts at 36 mg/kg (Table 3). Higher doses were also myelosuppressive, but lethality was excessive. Streptozotocin had essentially no effect on either total WBC or neutrophil counts.

Mitomycin C and the analogues M-83 and profiromycin caused marked reductions in total WBC and neutrophil counts at nonlethal doses (Table 4). The reduction in neutrophils produced by M-83 was somewhat less in magnitude than that caused by mitomycin C or porfiromycin.

Bleomycin caused minimal reductions in total WBC counts and no reductions in neutrophil counts (Table 5). The neutrophil counts of the mice treated with bleomycin at 139 mg/kg were actually higher than control on day 4. Vinblastine and vindesine caused marked neutropenia at MTD levels, whereas the neutropenic effect of vincristine was moderate at its MTD.

Table 1. Myelosuppressive effects of anthracyclines in mice

Drug	Dose mg/kg (IV)	% Δ on day 4		Deaths by day 14
		Total WBC	Neutrophils	
Adriamycin	32	−84	−97	10/10
	24	−76	−89	0/10
	18	−76	−80	0/10
	Vehicle	9	43	0/10
Aclacinomycin	37	−85	−91	9/10
	28	−89	−94	6/10
	21	−80	−84	0/10
	Vehicle	−18	−3	0/10
Carminomycin	3.2	−92	−98	0/10
	2.4	−86	−92	0/10
	1.8	−76	−94	0/10
	Vehicle	−7	26	0/10
Marcellomycin[a]	14.1[b]	−80	−95	3/10
	10.6	−54	−10	2/10
	7.9	−21	ND	0/10
	5.9	−33	ND	0/10
	Vehicle	−6	10	0/10

Animals: Male BDF_1 mice. *Treatment:* Single dose IV, highest dose is LD_{50}. ND, not done
[a] Maximum % Δ on day 7
[b] 1.33 × LD_{50}

Table 2. Myelosuppressive effects of platinum complexes in mice

Drug	Dose mg/kg (IV)	% Δ on day 4		Deaths by day 14
		Total WBC	Neutrophils	
Cisplatin	14	−44	79	3/10
	10.5	−30	24	2/10
	8	−17	ND	0/10
	Vehicle	−8	−5	0/10
Carboplatin	131	−53	−50	4/10
	98	−48	2	0/10
	74	−38	−3	1/10
	Vehicle	−14	36	0/10
Iproplatin	74	−82	−80	6/10
	56	−80	−72	2/10
	42	−52	−55	0/10
	Vehicle	−5	−2	0/10
Spiroplatin	7.5[a]	−44	85	2/10
	5.6	−25	ND	0/10
	4.2	−16	ND	0/10
	3.2	−2	ND	0/10
	Vehicle	10	22	0/10

Animals: Male BDF_1 mice. *Treatment:* Single dose IV, highest dose is LD_{50}. ND, not done
[a] Maximum % Δ on day 7
[b] 1.33 × LD_{50}

Table 3. Myelosuppressive effects of nitrosoureas in mice

Drug	Dose mg/kg (IV)	% Δ on day 4		Deaths by day 14
		Total WBC	Neutrophils	
BCNU	64	−80	ND	10/10
	48	−72	−71	9/10
	36	−62	−68	0/10
	Vehicle	−14	−10	0/10
Streptozotocin	208	−24	8	1/10
	156	−28	−20	0/10
	117	−10	ND	0/10
	Vehicle	0	−17	0/10

Animals: Male BDF_1 mice. *Treatment:* Single dose IV (BCNU IP), highest dose is LD_{50}.
ND, not done

The myelosuppressive effects of the 17 anticancer drugs in mice and man are summarized and correlated in Table 6. The mouse neutrophil count changes predicted correctly (true positive or negative) for the myelosuppressive effects in man of 13/17 drugs (76%). The predictions for marcellomycin, carboplatin, spiroplatin and cisplatin were false negatives according to our criteria.

Discussion

The mouse has been under evaluation for its ability to provide qualitative predictions for the organ toxicities of anticancer drugs in man [3, 4, 9–11]. The purpose of the current studies was to determine whether the mouse would be an effective animal model to screen anticancer drugs for

Table 4. Myelosuppressive effects of mitomycins in mice

Drug	Dose mg/kg (IV)	% Δ on day 4		Deaths by day 14
		Total WBC	Neutrophils	
Mitomycin C	8.6	−81	−97	8/10
	6.4	−74	−97	0/10
	4.8	−67	−91	0/10
	Vehicle	7	24	0/10
M-83	27	−82	−54	3/10
	20	−77	−58	2/10
	15	−69	−66	0/10
	Vehicle	−19	−32	0/10
Porfiromycin	37	−82	−97	8/10
	28	−74	−96	1/10
	21	−76	−82	0/10
	Vehicle	−14	45	0/10

Animals: Male BDF_1 mice. *Treatment:* Single dose IV, highest dose is LD_{50}

Table 5. Myelosuppressive effects of bleomycin and vinca alkaloids in mice

Drug	Dose mg/kg (IV)	% Δ on day 4		Deaths by day 14
		Total WBC	Neutrophils	
Bleomycin	185	−24	51	8/10
	139	−11	154	2/10
	104	−24	ND	2/10
	Vehicle	−7	−33	0/10
Vinblastine	22	−77	−99	10/10
	16.5	−69	−95	4/10
	12.4	−59	−98	1/10
	Vehicle	40	54	0/10
Vincristine	4.7	−42	−68	10/10
	3.5	−21	−46	2/10
	2.6	−1	18	0/10
	Vehicle	40	54	0/10
Vindesine	11.3	−70	−99	3/10
	8.5	−47	−98	0/10
	6.4	−37	−96	0/10
	Vehicle	29	−10	0/10

Animals: Male of BDF_1 mice. *Treatment:* Single dose IV, highest dose is LD_{50}. ND, not done

myelosuppressive effects. The total WBC count in mice is composed of about 75% lymphocytes and 20% neutrophils [12]. In our studies, a moderate to marked decrease in the total WBC count was not always associated with a corresponding decrease in the neutrophil count. Therefore, monitoring the total WBC count alone in mice does not provide a reliable indicator of the presence or absence of myelosuppression (neutropenia). The differential leukocyte count must be made and the specific drug effect on neutrophil counts determined.

The neutropenic effects observed the mice were in agreement with the myelosuppressive effects in man for 13/17 drugs tested. Importantly, there were no false positives. The peripheral neutrophil counts in mice failed to

Table 6. Correlation of myelosuppressive effects of anticancer drugs in mice and man

Myelosuppressive effect in man (rating)	Drugs	Neutropenic[a] effect in mice	Correlation
1. Dose-limiting (++)	Aclacinomycin [17][b]	++	T+
	Adriamycin [6]	++	T+
	BCNU [6]	++	T+
	Carboplatin [5]	0	F−
	Carminomycin [6]	++	T+
	Iproplatin [7]	++	T+
	Marcellomycin [13,15]	0	F−
	Mitomycin [6]	++	T+
	M-83 [16]	++	T+
	Porfiromycin [1]	++	T+
	Spiroplatin [20]	0	F−
	Vinblastine [6]	++	T+
	Vindesine [2]	++	T+
2. Occurs, but not dose-limiting (+)	Cisplatin [6]	0	F−
	Vincristine [6]	+	T+
3. Little or none (0)	Bleomycin [6]	0	T−
	Streptozotocin [6]	0	T−

[a] *Criteria:* based on %Δ in neutrophil counts at MTD (LD_{0-20}): ++ >65%↓; + = 35%−65%↓; 0 = <35%↓

[b] Reference for myelosuppressive effect in man

predict myelosuppression for cisplatin, carboplatin, and spiroplatin, indicating that this method is not useful for screening platinum complexes for myelosuppression. The reason for the failure to predict for platinum complexes is not known, but similar findings have been reported by others [9, 14]. The marked myelosuppressive effect of iproplatin adds to the mystery.

We evaluated the effects of these drugs on mouse neutrophil counts using single-dose treatment and fixed postdose blood sampling time points. It might have been more precise to optimize the postdose bleeding schedule for each drug to identify the nadir day and effect more specifically. However, preliminary testing with mitomycin C, Adriamycin, aclacinomycin, bleomycin, and BCNU indicated that the day 4 postdose determination of neutrophil counts would be sufficiently accurate for screening purposes. However, according to the results with marcellomycin, it is possible that use of day 4 data only could produce a false-negative result with a drug that causes delayed myelosuppression.

Identification of an equivalent dose for comparison of effects among the various drugs presented a challenge. The test doses of each drug were selected on the basis of the LD_{50} previously determined in unbled mice. However, as shown in the summary tables, this LD_{50} did not always predict well for the incidence of lethality in mice in the myelosuppression tests. Therefore, the mice in each test were observed for 14 days after dosing, and comparisons of myelosuppressive effects were made at the approximate maximum tolerated dose (LD_{0-20}) observed in each study.

The small proportion of neutrophils relative to the total WBC count in mice contributes to variability within groups and between bleedings in individual mice. In these studies the change in neutrophil counts in the vehicle-treated mice from day −2 (predose) to day 4 (postdose)

ranged from −33% to 54%. We have not been able to reduce this variability through control of certain other variables (mouse weight, time of day of bleeding and dosing, time between predose bleeding and dosing, acclimating mice to housing). For screening purposes, we continue to compare the postdose cell counts with the predose counts for each mouse rather than comparing the treated mice with the control mice that receive vehicle only. However, if the neutrophil counts in the vehicle-treated mice are reduced by more than 35% on day 4 the entire study is discarded.

If the platinum complexes are excluded, the predictions were correct for 12/13 drugs, or 92%. This demonstrates that measurement of anticancer drug effects on mouse peripheral neutrophil counts is a good predictor for clinical myelosuppression and can be used effectively to screen new anticancer drugs, with the exception of platinum complexes, for this toxicity. However, since the incorrect predictions were false negatives, it is critical that advanced evaluation methods be used for further testing of apparently less myelosuppressive agents. We currently test such drugs in a murine CFU-C assay [18] and a ferret hematology model [19] for advanced evaluations of their myelosuppressive effects.

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