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Quantifying the Carcinogenicity of Antineoplastic Drugs

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Abstract—*It has been well established that many of the drugs used in cancer therapy are themselves potentially carcinogenic. It is therefore important to quantify the carcinogenic risk associated with specific agents, and to investigate ways of predicting their risk from animal and* in virtro *studies. In this paper, an index of carcinogenic potency is defined, and applied to published data on acute non-lymphocytic leukemia following therapy with cytotoxic drugs used as single agents. Carcinogenic potency estimates for rats and mice are also obtained for* 15 *antineoplastic drugs, and the potency correlation between humans and rodents is examined for the five agents for which there are data in common. The broader implications for quantitative cancer risk prediction are discussed.*

INTRODUCTION

THE SUCCESS of multiple agent chemotherapy in the treatment of Hodgkin's disease has been unfortunately accompanied by the long-term complication of acute non-lymphocytic leukemia (ANLL), non-Hodgkin's lymphoma and possibly other cancers [1, 2]. Survivors of non-Hodgkin's lymphoma [3, 4], ovarian cancer [5–7], multiple myeloma [8, 9] and small cell carcinoma of the lung [10] have also experienced high rates of ANLL, which have been ascribed to antineoplastic therapy.

It appears that the risk of ANLL is associated with the alkylating agents, and studies of treated patients have specifically implicated chlorambucil [5, 11], cyclophosphamide [4, 6, 12], melphalan [7, 13], busulphan [14], treosulphan [15] and methyl-CCNU [16]. Combination therapies involving alkylating agents in conjunction with other drugs have also produced excesses of ANLL.

Many antineoplastic drugs have been tested for carcinogenicity in animal bioassays. In addition to tumors of the hematopoietic system, they have been shown to induce a wide range of solid tumors in treated animals. Some agents, such as procarbazine and *cis*-platinum, are well-established animal carcinogens, but cannot yet be evaluated in humans, either because they are rarely given alone, or because their use is too recent for the appropriate studies to have been made.

At present, the clinical use of antineoplastic drugs is mainly limited by acute complications. Nevertheless, many oncologists are now concerned about the long-term hazard of second tumors, particularly in the management of malignancies such as Hodgkin's disease, for which survival has been radically improved by combination chemotherapy which includes alkylating agents [17], or malignancies associated with a relatively low mortality such as early-stage breast cancer [18].

In this paper, we examine the quantitative relationship between the carcinogenicity of antineoplastic drugs in humans and their carcinogenicity in animal experiments. The immediate goal is to evaluate the predictive value of animal tests in assessing the risk of second cancer in patients treated with antineoplastic drugs. However, the results have implications for the more general problem of using animal carcinogenicity data in the prediction of human risk.

MATERIALS AND METHODS

Data sources

A literature search was conducted for published data on the carcinogenicity of antineoplastic drugs in humans and in experiments on rats and mice. The principal source used was the IARC series of Monographs on the Evaluation of Carcinogenic Risks to Humans [31], which was supplemented by

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Table 1. Carcinogenicity of antineoplastic drugs in humans: studies used in potency calculations

Drug	Primary disease	Number of patients	Number of cases of leukemia*	Reference
Busulfan	Lung cancer	69	4†	Stott *et al.* 1977 [14]
Chlorambucil‡	Polycythemia vera	141	16	Berk *et al.* 1981 [11]
	Ovarian cancer	71	2	Greene *et al.* 1982 [5]
Cyclophosphamide	Ovarian cancer	333	3§	Greene *et al.* 1986 [6]
Melphalan	Ovarian cancer	605	21§	Greene *et al.* 1986 [6]
	Breast cancer‖	5299	34¶	Fisher *et al.* 1985 [18]
Treosulphan	Ovarian cancer	553	8	Pedersen-Bjergaard *et al.* 1982 [15]
Semustine	Gastrointestinal cancer	2067	14**	Boice *et al.* 1983 [16]
Actinomycin D	Gestational trophoblastic tumors	600	0	Trapido, personal communication
Methotrexate	Gestational trophoblastic tumors	1013	0	Trapido, personal communication

*Unless otherwise specified, the leukemias were of the acute, non-lymphocytic type.
†Includes one case reported as 'atypical chronic myelomonocytic leukemia in transformation'.
‡Reported as 'acute leukemia'.
§Includes 'preleukemia'.
‖Non-alkylating agents (5-fluorouracil and methotrexate) were also given, in adition to melphalan.
¶Includes seven cases reported as 'myeloproliferative syndrome', and one case of chronic lymphoid leukemia.
**Includes seven cases of 'preleukemia' or 'acute myelodysplastic syndrome'.

articles which had appeared on specific agents since the publication of the corresponding monograph. The published studies were screened for suitability, according to the following criteria.

We required that human studies be based on a clearly defined group of patients suffering from the same initial cancer, and followed up for the occurrence of leukemia. We further required that the study provide information on the carcinogenicity of single antineoplastic agents, that information on dosage be available, and that some measure of the incidence of second cancer by calculable. For two agents, actinomycin D and methotrexate, we used unpublished data, since no other data were available on these drugs alone.

Carcinogenicity bioassays on rats or mice were accepted if they satisfied the criteria defined by Gold *et al.* [19]. Briefly, treated animals were chronically exposed to a single agent, via a route which could result in whole body exposure, starting early in life and for at least a quarter of the animal's normal lifespan. Also, there were at least five animals treated, and a concurrent control group. A summary of all such experiments reported up to July 1981 has been published by Gold *et al.* [19], and recently updated [20]. A large number of the experiments were carried out under the US National Toxicology Program (NCI/NTP).

Endpoints considered

For humans, we only evaluated the risk associated with leukemia since there is very little information on the association between specific antineoplastic drugs and other cancers in humans. If a study only reported on acute myelogenous leukemia or acute leukemia, this was accepted as the endpoint. Some studies also reported on 'preleukemia', 'myeloproliferative syndrome' or 'acute myelodysplastic syndrome'.

For animal studies, we considered as endpoints all tumors, and all tumors of the hematopoietic system. If a study only reported on a specific hematopoietic tumor, this was accepted as the endpoint.

Quantitative description

For human data, the carcinogenicity was summarized by the cumulative 10-year incidence of leukemia per unit total dose, calculated by dividing the cumulative incidence estimated in the study by the mean (or median) cumulative dose, averaging over all patients under study. When 10-year cumulative incidence was not available, it was estimated under the assumption of proportionality: thus if x-year cumulative incidence I_x was given, the 10-year incidence was estimated as

$$I_{10} = \frac{10}{x} I_x.$$

For animal experiments, we used the TD_{50} [21], which is the dose estimated to reduce the proportion of tumor-free animals by 50%. The TD_{50} can be calculated for single sites, or combined sites, in which case a 'tumor-free' animal is one which does

Table 2. Carcinogenicity of antineoplastic drugs in humans: leukemia in cancer survivors

Drug	Time period (years): mean (or median)	Time period (years): for incidence calculation	Cumulative incidence (%)	Mean (or median) total dose (g)	Potency index (10-year incidence per gram total dose
Busulfan	—*	8	3.3	3.2	1.3
Chlorambucil					
Berk *et al.* [11]	5.4	8	25	7.5	4.2
Greene *et al.* [5]	3.7	7	5.7	4.4†	1.8
Cyclophosphamide	2.8	10	5.4	19.5	0.28
Melphalan					
Greene *et al.* [6]	3.0	10	11.2	0.60	18.7
Fisher *et al.* [18]	—*	10	1.7	0.52	3.3
Treosulphan	2.1	5	7.6	140	0.11
Semustine	3.0	6	4.0	1.6†	4.2
Actinomycin D	9.0	9	0.0	0.008	—
Methotrexate	10.0	10	0.0	0.56	—

*Unavailable.
†Protocol dose.

Table 3. Available experiments on the carcinogenicity of antineoplastic drugs in mice and rats

	Mouse		Rat	
	Female	Male	Female	Male
Actinomycin D			P 4	P 4
Chlorambucil	P 4	P 4		P 4
Cyclophosphamide	P 4	P 4	P 3	P 3
Dacarbazine	P 4	P 4		
5-Fluorouracil			V 1	V 1
Isophosphamide	P 5	P 5	P 5	P 5
Melphalan	P 4	P 4	P 4	P 4
6-Mercaptopurine				V 1
Methotrexate	O 2	O 2	P 3	V 1
Mitomycin C			P 4	P 4
Busulphan				G 1
Nitrogen mustard				V 1
Procarbazine	P 5	P 5	P 5	P 5
Thio-TEPA	P 5	P 5	P 5	P 5
Vinblastine				V 1

P = intraperitoneal injection; V = intravenous injection; G = gavage; O = oral; 1 = Schmähl and Osswald [22]; 2 = Rustia and Shubik [23]; 3 = Schmähl and Habs [24]; 4 = Weisburger [25]; 5 = NCI/NTP.

not have a tumor at any of the sites being combined. The TD_{50}s and confidence limits were abstracted from the database reported by Gold *et al.* [19]. Note that for the TD_{50}, a smaller value indicates a more potent carcinogen, while for the human index, larger values arise for stronger carcinogens.

RESULTS

Human data

Table 1 gives the antineoplastic drugs for which appropriate human data were available, as well as the initial cancer, the number of patients studied, and the number of leukemias arising as second malignancies. Table 2 gives further details on the follow-up interval following diagnosis of the first cancer, the cumulative incidence of leukemia, the average total dose of drug given and the estimated potency index. The cumulative incidence is over the time period reported in the second column. The average total dose is an average over all patients, regardless of the duration of follow-up. In two studies, only protocol doses were reported, rather than the average of the true doses administered to the cancer patients.

It is clear from Table 2 that there is considerable variation in both the leukemia incidence and the average total dose, by drug. From the estimates in the last column, melphalan emerges as the most potent leukemogen in humans (although there is a considerable discrepancy between the two studies cited), followed by chlorambucil and semustine, and then busulfan. Cyclophosphamide and treosulphan are almost certainly leukemogenic, since they produce statistically significant excess of acute leukemia, but are much weaker. Actinomycin D does not appear to produce leukemias at the doses used, which are limited by its toxicity. Methotrexate is probably not leukemogenic, and if it is, it is several orders of magnitude weaker than melphalan. The 95% upper confidence limit on the potency estimates for actinomycin D and methotrexate were 7.2 and 0.58, respectively. Among the leukemogenic agents, there is a potency range of about 170-fold.

Animal data

Table 3 summarizes the data sources [22–25] for the experiments used by Gold *et al.* [19] in the calculation of TD_{50}s in mice and rats. The table also indicates the route of exposure, which is most often by intraperitoneal injection. Table 4 gives the all-

Table 4. Carcinogenicity of antineoplastic drugs in mice and rats. TD_{50} *for all tumors* (mg/kg/*day*)

	Mouse		Rat	
	Female	Male	Female	Male
Actinomycin D			0.0012	0.00071
Chlorambucil	0.060	0.13		0.71
Cyclophosphamide	1.8	3.8	1.3	2.9*
Dacarbazine	0.81	1.5		
5-Fluorouracil				7.0†
Isophosphamide	3.6	1.8†	0.38	1.0
Melphalan	0.10	0.11	0.078	0.047
6-Mercaptopurine				58*
Methotrexate	48.0*	2.0†	0.41†	0.55†
Mitomycin C			0.0011	0.00073
Busulphan				0.12*
Nitrogen mustard				0.023
Procarbazine	0.19	0.46		
	0.39	1.7	0.33	0.19
Thio-TEPA	0.085	0.069	0.040	0.033
Vinblastine				0.038†

*Dose–response curve had a positive slope, which was not significantly different from zero at the 0.05 level of significance.
†Dose–response curve had a negative slope; the value given is the lower limit of the 99% confidence interval for the TD_{50}.

Table 5. Carcinogenicity of antineoplastic drugs in mice and rats. TD_{50} *for tumors of the hematopoietic system** (mg/kg/*day*)

	Mouse		Rat	
	Female	Male	Female	Male
Actinomycin D				0.020
Chlorambucil	0.71	0.43		1.4†/1.7
Cyclophosphamide	7.1	8.7†		
Dacarbazine	8.0	13‡		
5-Fluorouracil				
Isophosphamide	5.1§			
Melphalan	0.70‖	0.30	1.5‖	0.36
6-Mercaptopurine				
Methotrexate				
Mitomycin C				0.039¶
Busulphan				
Nitrogen mustard				
Procarbazine	0.76**	3.3**		2.1**
Thio-TEPA	0.22††	0.24**		0.22**
Vinblastine				

*Unless otherwise specified, the tumors were all lymphosarcomas.
†Leukemia.
‡Lymphatic leukemia.
§Histiocytic lymphoma.
‖Dose-response curve had a positive slope, but was not significantly different from zero at the 0.05 level of significance.
¶Liver lymphosarcoma.
**Leukemia + lymphoma.
††Lymphocytic leukemia + lymphoma.

site TD_{50}s, and Table 5 gives the TD_{50}s for tumors of the hematopoietic system.

Thio-TEPA and chlorambucil emerged as the most potent overall carcinogens among the agents tested in mice. They were followed by melphalan, and then procarbazine and dacarbazine. Cyclophosphamide and isophosphamide were the weakest drugs with significant carcinogenic activity, and methotrexate was not a carcinogen in mice. Among the carcinogens, there was an approximately 50-

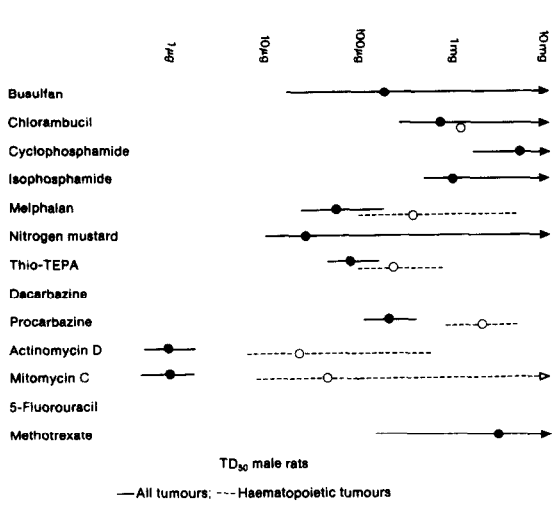

Fig. 1. TD_{50}*s with 99% confidence intervals for male rats. —— All tumors; --- hematopoetic system tumors.*

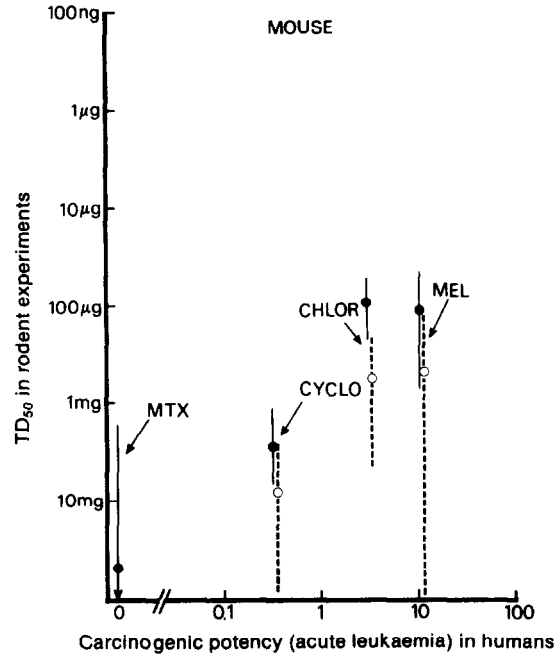

Fig. 2. TD_{50}*s with 99% confidence intervals for carcinogenic potency in mice (y-axis) plotted against leukemogenic potency in humans (x-axis). —— All tumors; --- hematopoietic system tumors. Abbreviations: CHLOR = chlorambucil; CYCLO = cyclophosphamide; MEL = melphalan; MTX = methotrexate.*

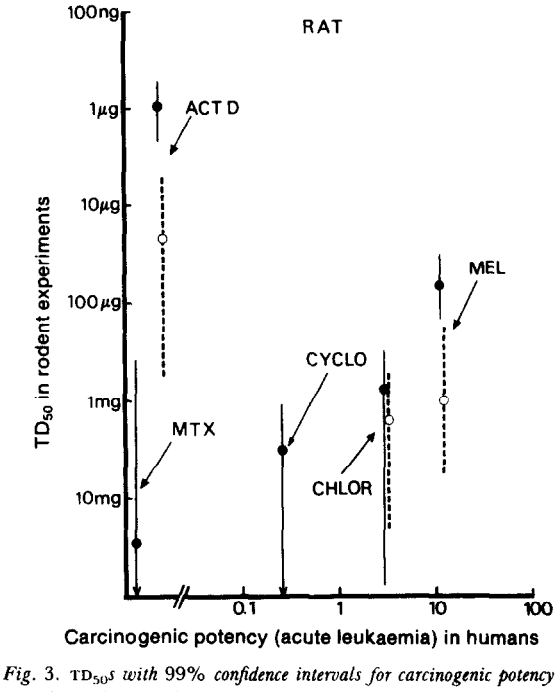

Fig. 3. TD_{50}*s with 99% confidence intervals for carcinogenic potency in rats (y-axis) plotted against leukemogenic potency in humans (x-axis). —— All tumors; --- hematopoietic system tumors. Abbreviations: ACT D = actinomycin D; CHLOR = chlorambucil; CYCLO = cyclophosphamide; MEL = melphalan; MTX = methotrexate.*

fold range in potency.

In rats, actinomycin D and mitomycin C were by far the most potent carcinogens when tumors at all sites are considered. They are followed by nitrogen mustard (which was only tested in males), thio-TEPA and melphalan. Procarbazine was somewhat weaker than melphalan, and it was followed by chlorambucil (only tested in males), isophosphamide and cyclophosphamide. The remaining agents did not increase the overall tumor incidence in rats. The potency range in rats was over 1000-fold, but is reduced to less than 40-fold if actinomycin D and mitomycin C are excluded.

The pattern for tumors of the hematopoietic system was rather similar, as shown in Table 5. Again, thio-TEPA, chlorambucil and melphalan were the most potent agents in mice. They were followed by procarbazine, isophosphamide (only for females), cyclophosphamide and dacarbazine. In rats, a comparison was only possible for males. Actinomycin D and mitomycin D were again the most potent agents, followed by thio-TEPA, melphalan, chlorambucil and procarbazine.

Figure 1 shows the all-site and hematopoietic system potency estimates with 99% confidence intervals, for male rats. The relationship between potency estimates in rodents and humans is shown in Fig. 2 for mice and Fig. 3 for rats. In each of the two figures, 99% confidence intervals for the TD_{50}s at all sites, and for tumors of the hematopoietic system are plotted against human potency estimates based on leukemia. In these plots, the TD_{50}s are the average over males and females, if potencies for both sexes are available, and for chlorambucil and melphalan, the potencies in humans are averages over the two studies available in each case. Only drugs with data on both humans and mice or rats appear on each plot.

DISCUSSION

In this paper, we have summarized the quantitative information available on the carcinogenicity of antineoplastic drugs in humans, and in experiments

on rats and mice. The purpose of this exercise has been to evaluate the limitations of the data, to rank the drugs according to carcinogenic potency, and to examine the correlation between the potency estimates in rodents and humans. In the discussion below, we consider each of these aspects, and discuss more general implications of the results for the quantitative prediction of carcinogenic risk in humans.

Animal data

For the animal carcinogenicity experiments, the TD_{50}s were readily available from the comprehensive database published by Gold *et al.* [19, 20]. The criteria used by these authors and outlined in the 'methods' for acceptance of experiments into the database ensure a level of uniformity in the type and quality of experiments analyzed. Nevertheless, there are various assumptions and simplifications which must be made, either implicitly or explicitly, if the TD_{50} values are to be viewed as comparable across experiments (see Peto *et al.* [21] for a more detailed description of the problems in estimating and interpreting the TD_{50}).

The experiments come from several sources, and differ substantially with regard to strain of rat or mouse, route of exposure, numbers of animals tested, and the degree of pathological investigation undertaken. Another important difference is the type of data available. Only the NCI/NTP experiments provided information on time-to-tumor and survival for each animal, which is preferable in the estimation of the TD_{50} [21], since it avoids the downward bias (due to toxicity) of crude cumulative incidence data.

Although the TD_{50} often falls within the range of observation in animal experiments, several of the estimates reported here are based on experiments where the highest observed level of tumor incidence was substantially below the 50% required for the TD_{50}, so that linear expolation was required. Indeed, more generally, the calculation of the TD_{50} implies that there is a linear relationship between dose and tumor incidence. However, for three of the agents (isophosphamide, procarbazine and thio-TEPA), there was strong evidence of upward curvature (see Gold *et al.* [19]). It is notable that for all three agents, the estimate was based on time-to-tumor data. A recent paper comparing time-to-tumor and crude cumulative incidence data in the calculation of TD_{50} demonstrated that upward curvature is often badly underestimated from the latter type of data [20]. For more details on differences among the experiments reported here, see Gold *et al.* [19] or the original publications.

Although it was possible to establish rankings on the basis of the TD_{50}s, it is important to bear in mind the range of uncertainty associated with most of the estimates (see Fig. 1). For example, there is considerable overlap among the 99% confidence intervals for the TD_{50}s of the mustards, chlorambucil, cyclophosphamide and isophosphamide in male rats, although chlorambucil appears by far the most potent of the three agents in female mice. For the tumors of the hematopoietic system, a number of the 99% confidence intervals covered nearly two orders of magnitude even when the dose–response was significant at the 0.01 level, due to the small number of tumors observed. In general, the TD_{50}s estimated for these tumors were greater than the all-site TD_{50}s, although the rankings among chemicals were more or less preserved.

Human data

Estimating carcinogenic potency from human observations is an even more tenuous undertaking than it is for animal experiments. There is not yet agreement on an appropriate index of carcinogenicity, let alone on the methods which should be used to estimate it. In this paper, we have defined an index which is comparable to the inverse of the TD_{50}, namely the excess cumulative incidence of cancer over a 10-year period, per unit total dose of the drug. It has the advantage of being based directly on observed exposure levels, rather than extrapolated outside the range of the observed data. Although the index is defined on an individual level, it has been necessary to estimate it from the ratio of cumulative incidence to average total dose in the study population. Ideally the index would be estimated using individual records for each patient, in which dose, survival time and the time to occurrence of a second cancer are given. This level of detail is so far unavailable for all of the drugs considered here.

Like the TD_{50}, the human potency index is calculated assuming that a single parameter, effectively the slope of the dose–response curve, can describe the carcinogenic potency of a drug. However, it is not possible to examine this assumption without data on the cancer incidence at several dose levels. It has also been necessary to assume that, for a fixed total dose the excess tumor incidence as a consequence of therapy is constant over the 10-year period following the start of therapy. If data were available on incidence in successive time periods following therapy, the assumption could also be checked.

Apart from these technical difficulties, there are other important factors which have not been taken into account in the ranking of potency in humans. These include the age and sex composition of the patient groups under study, and the nature of their primary cancer. All of these factors could play a role in the subsequent risk of leukemia.

An alternative index which could have been used for potency in humans is the cumulative leukemia incidence per daily dose rate. However, the dose rate is not given in most of the reports used here, even as an average. In fact, the definition of dose rate in humans is complicated by the long breaks which may occur between episodes of treatment. As a compromise we calculated the cumulative incidence per unit dose per month follow-up time, by dividing the cumulative incidence per unit total dose by the mean (or median, if the mean was unavailable) follow-up time in the study. For actinomycin D and methotrexate, which are given over very short time periods, this index is not meaningful. For the other agents, it gave rise to potency rankings almost identical to the first index.

Correlation between carcinogenic potency of antineoplastic drugs in humans and rodents

The ultimate goal in ranking the potency of the antineoplastic drugs in animals and humans is to study their correlation. If there is a strong relationship between animal and human potencies, then animal studies can be reliably used to predict which drugs present a greater carcinogenic risk when used in cancer therapy. The results displayed in Figs. 2 and 3 are somewhat encouraging in this regard. Among the three nitrogen mustard compounds, cyclophosphamide, chlorambucil and melphalan, the potency rankings are very similar in humans and rodents, whether all tumors or only those of the hematopoietic system are considered. A substantial outlier is actinomyin C, which is the most potent rat carcinogen among the drugs considered, but apparently does not produce an excess of leukemia in humans. This discrepancy is probably due to differences in mode of action between the nitrogen mustards, which alkylate DNA, and actinomycin D, which binds to DNA by intercalation [26].

A major question in studying potency correlations across species is the role of toxicity. Bernstein *et al.* [27] have shown that once the dose to be tested is fixed in animal experiments, generally on the basis of acute toxicity, there is only about a 30-fold range in which the potency estimate could fall. The correlation of carcinogenic potency between rats and mice could then be explained simply by a strong correlation between the corresponding LD_{50}s, and there may be a similar relationship between the animal LD_{50}s and the doses used in cancer therapy. This does not rule out the possibility that there is a true relationship between carcinogenic potency and toxicity. However, it does limit the nature of the conclusions that can be drawn from studies of potency correlation.

A striking feature of Figs. 2 and 3 is the paucity of drugs for which both humans and rodent potency estimation can be made. The absence of good human data is perhaps less surprising than the lack of reliable animal carcinogenicity experiments for some of these widely used antineoplastic drugs.

Quantitative risk prediction for humans

The antineoplastic agents represent a unique group of carcinogens, in that humans are intentionally exposed to them, at doses which are carefully measured. Although fundamentally an issue of clinical importance, the quantitative study of drug carcinogenicity has wider implications for predicting risk to humans from *in vitro* and animal assays. Previous studies [28, 29] which have attempted to investigate the relationship between carcinogenic potency in humans and animals have used disparate classes of chemicals such as aflatoxin and aromatic amines, for which human exposure data are very crude. Indeed, the publication dates of most of the studies summarized in Table 1 indicate that an exercise of the kind reported here has only recently become feasible. We are currently carrying out a large retrospective study of leukemia following Hodgkin's disease, ovarian cancer and testicular cancer, which should add additional quantitative information on drug carcinogenicity [30].

For the limited number of agents available for study, we have observed a reasonable correlation in potency ranking. However, risk prediction requires that the correlation be established quantitatively, and in this regard, the available data are too weak to allow any conclusions to be drawn. On the basis of the three nitrogen mustard derivatives in Figs. 2 and 3, the potency range in humans is more than two orders of magnitude, whereas in mice and rats, it is closer to one. There could be a number of reasons why the scales are not strictly comparable. The leukemia data in humans relate to a relatively short duration of exposure and follow-up time, while the animal studies generally involve entire lifetimes. Thus, differences in the rate at which the drugs exert their carcinogenic effect would tend to be accentuated in the human estimates, but diluted in the animal potencies. Other reasons for the difference could be the tumor types being considered (leukemia, generally ANLL, in humans, as compared with any hematopoietic cancer in the rodents) and the choice of daily dose per unit body weight, rather than, say, surface area, for the TD_{50}.

Clearly an expanded database of the kind presented here will be very useful in resolving the uncertainties in quantitative carcinogenic risk prediction.

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