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# Mouse and Large-Animal Toxicology Studies of Twelve Antitumor Agents: Relevance to Starting Dose for Phase I Clinical Trials

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***Summary.*** *Large-animal toxicology is presently used to establish a starting dose for clinical trials with new cancer chemotherapeutic agents. The relevance of dog, monkey, and mouse data for Phase I clinical trials has been retrospectively analyzed with twelve diverse agents (chlorozotocin, maytansine, anguidine, tritylcysteine, piperazinedione, Baker's antifol, thalicarpine, 3-deazauridine, gallium nitrate, cis-dichlorodiammineplatinum(II) (DDP), 4'-(9-acridinylamino)methanesulfon-m-anisidide (AMSA), and N-phosphonacetyl-L-aspartic acid (PALA). Schedules studied clinically included a daily × 5 schedule and a single dose schedule (three drugs), a daily × 5 schedule only (three drugs), and a single dose schedule only (six drugs). One-third of the toxic dose low (TDL) in the most sensitive large-animal species (dog or monkey), expressed in mg/m², was a tolerable starting dose in humans in all instances for the schedules employed. The number of dose escalation steps to reach the human maximum tolerated dose (MTD), according to the commonly used Fibonacci dose escalation scheme, varied from 2 to more than 12. Had one-third the $LD_{10}$ in mice, expressed in mg/m² been applied, this would also have yielded safe starting dose levels, and would actually have required a lesser number of dose escalations to reach the human MTD. This analysis confirms that mouse data may be quite useful in determining safe starting doses for Phase I trials with anticancer chemotherapeutic agents.*

## Introduction

Exploration of efficacy of anticancer chemotherapeutic agents usually takes place at a dose approaching the maximum tolerated dose (MTD), which may be defined as the highest dose consistent with tolerable and reversible side effects at a given schedule in a specific population of patients. Phase I clinical trials are carried out to identify the MTD and the dose-limiting toxicities of these agents. Among the most important characteristics of these clinical trials are the initial starting dose, the method of dose escalation, the schedules and methods of drug administration, and the study of pharmacokinetic parameters.

Animal toxicologic studies form the basis for defining the initial starting doses in clinical trials, and presumably also provide information about possible acute and cumulative toxic effects to be encountered. A rigorous protocol has been developed by the Laboratory of Toxicology, Division of Cancer Treatment, National Cancer Institute, for these purposes (Prieur et al., 1973). This protocol has involved essentially two large-animal species, the beagle dog and the rhesus monkey. Recently, toxicologic studies in mice have been added. In large animals, four dose levels are defined: HNTD (highest nontoxic dose), TDL (toxic dose low), TDH (toxic dose high), and LD (lethal dose). HNTD is the highest dose at which no hematologic, chemical, clinical, or morphologic drug-induced alterations occur; doubling this dose produces alterations in at least one of these parameters. TDL is the lowest dose that produces these alterations, and doubling the TDL does not produce lethality. TDH is the dose that produces these alterations, and doubling the TDH does produce lethality. LD is the lowest dose that produces drug-induced death in any animal during the treatment or observation period. These dose levels are determined on two basic schedules (single dose and daily × 5). Other dose schedules are also investigated (Table 1). $LD_{10}$ is the dose of drug that kills 10% of the non-tumor-bearing mice during a period of observation. Whereas two paired animals are used in large-animal studies, groups of 10 mice at each dose level are used to determine points on a probit scale (Goldin et al., 1979).

Starting doses for Phase I clinical trials have usually been chosen from the dose corresponding to one-third of

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**Table 1.** Protocol toxicology studies

| | |
|---|---|
| Study I. | Single dose – dogs |
| Study II. | Five consecutive daily treatments – dogs |
| Study III. | Five consecutive daily treatments – monkeys |
| Study IV. | Five consecutive daily treatments, 9 days rest, repeated for three treatment periods – dogs |
| Study V. | Schedule dependency studies – dogs |
| | a. 48-h IV infusions weekly for 6 weeks |
| | b. Treatment every 6 h for 48 h, weekly for 6 weeks |
| | c. Single dose, once weekly for 6 weeks |
| | d. 10 consecutive daily treatments |
| Study VI. | $LD_{10}$, $LD_{50}$, $LD_{90}$ – mice |

**Table 2.** Modified Fibonacci search scheme for escalation of drug dosage

| Dose | Percentage increase above preceding dose level |
|---|---|
| n | – |
| 2n | 100 |
| 3.3n | 67 |
| 5n | 50 |
| 7n | 40 |
| 9n | 33 |
| 12n | 33 |
| 16n | 33 |

the TDL of the most sensitive large-animal species in units of mg/m$^2$ body surface area. Dose escalations are usually carried out according to a modified Fibonacci search scheme with decreasing incremental steps (Table 2).

In this study we retrospectively analyzed large-animal and small-animal toxicologic data on twelve drugs introduced into clinical testing between 1972 and 1978 and for which Phase I clinical trials were completed according to similar methodologies. Correlations were then carried out between all quantitative toxicologic information in animals, and that obtained in humans. Our purpose was to determine the relevance of large animal as against mouse toxic dose levels for Phase I clinical trials. Qualitative aspects such as the types of toxicity reportedly predicted by acute or chronic animal toxicology were not considered in this analysis.

## Materials and Methods

Phase I clinical trials with chlorozotocin, maytansine, anguidine, tritylcysteine, piperazinedione, Baker's antifol (BAF), thalicarpine, 3-deazauridine, gallium nitrate, *cis*-dichlorodiammineplatinum(II) (DDP), 4′-(9-acridinylamino)methanesulfon-m-anisidide (AMSA), and *N*-phosphonacetyl-L-aspartic acid (PALA) were carried out under the sponsorship of the Division of Cancer Treatment, National Cancer Institute. Detailed animal toxicologic and clinical information is available for all these drugs, which represent a variety of different structures and properties. Data for the dog, monkey, and mouse was provided by the Laboratory of Toxicology. In three instances, mouse schedules had to be converted to conform to the actual schedule used in humans (Table 3, footnote d). The MTD we used corresponds to the dose and schedule used or recommended for Phase II trials in solid tumors. This is an arbitrary definition, since patients considered as 'good risk' with no prior treatment and no organ dysfunction, and also leukemic patients, often receive higher doses of drug without life-threatening toxicity. We compared one-third the TDL for the most sensitive large-animal species and one-third the $LD_{10}$ in mice with the human dose eventually used in clinical studies. The number of dose escalation steps required to reach the human MTD according to the Fibonacci method was then calculated. In addition, we looked at mouse $LD_{10}$ data from a wider number of drugs and examined their variability and implications for the number of dose escalation steps. The basis for this additional data is provided elsewhere (Guarino et al., 1979).

## Results

Table 3 shows the schedule, human MTD, large animal TDL, and mouse $LD_{10}$ for the 12 drugs compared. The TDL for the single-dose schedule is for the dog, since only that species is used for single-dose toxicology studies. The exception is thalicarpine, for which single-dose studies were carried out only in the monkey. Drugs for which it is possible to make a most sensitive large animal species comparison are those for which toxicologic studies were carried out on the daily × 5 schedule. The dog is the most sensitive large-animal species for three drugs (anguidine, piperazinedione, and BAF); the monkey for one drug (tritylcysteine), and both the dog and monkey are equally sensitive for two drugs (3-deazauridine and DDP).

Table 4 gives the ratio of human MTD to one-third TDL for large animals. When this ratio is greater than 1, the choice of one-third TDL constitutes a tolerable initial starting dose in humans. This was the case for all 12 drugs and all schedules studied, confirming the relevance of these data to the clinical situation (Goldsmith et al., 1975). Also shown is the ratio of human MTD to one-third the mouse $LD_{10}$. This ratio is less than 1 for anguidine and piperazinedione at the daily × 5 schedule, and the initial starting dose of one-third the mouse $LD_{10}$ could have induced unacceptable toxicity. However, it should be noted that the ratio has been calculated from daily × 9 $LD_{10}$ data. If the single-dose $LD_{10}$ data had been used, the ratios would have been greater than 1 (4.5/3 for anguidine, and 3.0/1.9 for piperazinedione). This points out the pitfalls in schedule conversions, and the need for actual experimental data for these calculations. In these two instances one has to assume that the 'true' ratio for daily × 5 $LD_{10}$ data would have fallen somewhere in between the ratios calculated from the sin-

**Table 3.** Human clinical dose (MTD), toxic dose low (TDL), and $LD_{10}$[a]

| Drug | Schedule | MTD[b] (human) | TDL (large animal) | | $LD_{10}$ (mouse) |
|---|---|---|---|---|---|
| Chlorozotocin | d × 1 | 200 | 30[c] | (dog) | 74 |
| Maytansine | d × 1 | 2 | 0.3 | (dog) | 1.26 |
| Anguidine | d × 5 | 4.5 | 0.62 | (dog) | 27.5[d] |
| | d × 1 | > 10 | 1.26 | (dog) | 45 |
| Tritylcysteine | d × 5 | > 500 | 19 | (monkey) | – |
| Piperazinedione | d × 5 | 3 | 0.94 | (dog) | 11.9[d] |
| | d × 1 | 9 | 7.5[c] | (dog) | 28.8 |
| BAF | d × 5 | 250 | 12.5 | (dog) | 308[d] |
| | d × 1 | 500 | 100 | (dog) | 177 |
| Thalicarpine | d × 1 | 1,100 | 525 | (monkey) | 615 |
| 3-Deazauridine | d × 5 | 1,200 | 1,250 | (both) | 567 |
| Gallium nitrate | d × 1 | 700 | 160 | (dog) | 150 |
| DDP | d × 5 | 20 | 7.75 | (both) | 11.1 |
| AMSA | d × 1 | 120 | 31.2 | (dog) | 91.2 |
| PALA | d × 1 | 5,000 | 2,400 | (dog) | 3,237 |

[a] Doses in $mg/m^2$
[b] MTD data are from Investigational Drug Branch IND files
[c] This represents the TDH; no TDL was determined
[d] Converted from d × 9 schedule

**Table 4.** Number of dose escalations required in humans by modified Fibonacci search scheme starting at one-third TDL or $LD_{10}$

| Drug | Schedule | MTD (1/3 TDL) | MTD (1/3 $LD_{10}$) | No. of escalations with | |
|---|---|---|---|---|---|
| | | | | 1/3 TDL (large animal) | 1/3 $LD_{10}$ (mouse) |
| Chlorozotocin | d × 1 | 20 | 8 | 8 | 5 |
| Maytansine | d × 1 | 20 | 4.8 | 8 | 3 |
| Anguidine | d × 5 | 21.4 | 0.5 | 8 | < 1 |
| | d × 1 | > 23.8 | > 0.7 | > 8 | – |
| Tritylcysteine | d × 5 | > 79 | – | >12 | – |
| Piperazinedione | d × 5 | 9.7 | 0.7 | 6 | < 1 |
| | d × 1 | 3.6 | 0.9 | 3 | < 1 |
| BAF | d × 5 | 59.5 | 2.4 | 12 | 2 |
| | d × 1 | 15 | 8.5 | 7 | 5 |
| Thalicarpine | d × 1 | 6.3 | 5.4 | 4 | 4 |
| 3-Deazauridine | d × 5 | 2.9 | 6.4 | 2 | 4 |
| Gallium nitrate | d × 1 | 13.1 | 14 | 7 | 7 |
| DDP | d × 5 | 7.7 | 5.4 | 5 | 4 |
| AMSA | d × 1 | 11.5 | 3.9 | 6 | 3 |
| PALA | d × 1 | 6.2 | 4.6 | 4 | 3 |

gle dose and daily × 9 $LD_{10}$ data. For piperazinedione at the single-dose schedule, one-third the $LD_{10}$ is roughly equivalent to the human MTD. This latter dose has been frequently exceeded in Phase II trials.

Table 4 also compares the number of Fibonacci dose escalation steps that would have been required to reach the human MTD if the initial starting dose were one-third of the large animal TDL or one-third of the mouse $LD_{10}$. It has been considered that optimally a Phase I study should not require more than six dose escalation steps, and the ratio of human MTD to one-third of the TDL for large animals and to one-third of the $LD_{10}$ in mice may be regarded as a measure of the efficiency of reaching a human MTD from the selected starting dose. If one-third of the TDL of the large animal had been used as the initial starting dose in humans, the number of Fibonacci dose escalation steps required to reach the human MTD would have varied from two to

**Table 5.** Effect of variability of mouse toxicity data on estimated number of Fibonacci steps in clinical trials[a, b]

| Drug | Schedule | Mouse $LD_{10}$ | | % Difference | Change in no. of Fibonaci steps |
|---|---|---|---|---|---|
| | | Lowest | Highest | | |
| Porfiromycin | d × 1 | 75 | 78 | 4 | 0–1 |
| 6-Mercaptopurine | d × 7 | 129 | 141 | 7 | 0–1 |
| Nitrogen mustard | d × 1 | 9.3 | 10.5 | 13 | 0–1 |
| Aniline mustard | d × 7 | 15.6 | 17.7 | 14 | 0–1 |
| Nitromin | d × 7 | 57 | 66 | 16 | 0–1 |
| FUDR | d × 7 | 327 | 384 | 17 | 0–1 |
| Cycloleucine | d × 7 | 222 | 264 | 19 | 0–1 |
| Cyclophosphamide | d × 5 | 63 | 78 | 24 | 0–1 |
| Mitomycin C | d × 7 | 4.8 | 6.0 | 25 | 0–1 |
| DTIC | d × 1 | 1,878 | 2,568 | 37 | 0–1 |
| Actinomycin D | d × 7 | 0.21 | 0.29 | 40 | 0–1 |
| Adriamycin | d × 1 | 30 | 42 | 40 | 0–1 |
| BCNU | d × 1 | 63 | 90 | 43 | 0–1 |
| | d × 7 | 42 | 63 | 50 | 0–1 |
| Thio-TEPA | d × 7 | 48 | 72 | 50 | 0–1 |
| Mitomycin C | d × 1 | 18 | 27 | 50 | 0–1 |
| Methotrexate | Weekly | 126 | 216 | 71 | 0–1 |
| L-PAM | d × 1 | 33 | 57 | 73 | 0–1 |
| Nitrogen mustard | d × 7 | 1.2 | 2.1 | 75 | 0–1 |
| Azaserine | d × 7 | 33 | 63 | 91 | 0–1 |
| TIC-mustard | d × 1 | 786 | 1,560 | 99 | 0–1 |
| Camptothecin | d × 1 | 174 | 423 | 143 | 1–2 |
| Cyclophosphamide | Weekly | 147 | 441 | 200 | 1–2 |
| Picolinaldehyde | d × 5 | 273 | 867 | 218 | 1–2 |
| Vincristine | d × 1 | 2.5 | 8.1 | 227 | 1–2 |
| 5-Fluorouracil | d × 1 | 186 | 720 | 287 | 2–3 |
| Pseudourea | d × 5 | 42 | 180 | 329 | 2–3 |
| Daunomycin | d × 1 | 16.2 | 87 | 437 | 3–4 |

[a] Mouse data from Laboratory of Toxicology files. $LD_{10}$ is given in $mg/m^2$ for indicated number of consecutive days

[b] Data from Guarino et al. (1979)

more than twelve. A human MTD was not established with tritylcysteine, because thrombophlebitis prevented escalation of the dose beyond 500 $mg/m^2/d \times 5$, but at least thirteen dose escalation steps would have been required to reach this dose level. Also, no human MTD was established for anguidine on the single-dose schedule, but at least nine dose escalation steps would have been required to reach the clinically tolerated dose level of 10 $mg/m^2$ by using one-third of the large-animal TDL as a starting dose. If one-third the mouse $LD_{10}$ had been used as the initial starting dose in humans, however, the number of Fibonacci dose escalation steps that would have been required to reach the human MTD ranges from less than one (anguidine and piperazinedione) to seven (gallium nitrate).

In the review of mouse $LD_{10}$ data for a large number of other drugs, some substantial variations have become apparent. Table 5 indicates the effect of such variations on these Fibonacci dose escalation steps if mouse data were the primary determinant for starting doses in clinical trials.

Although there is a range of 4% (porfiromycin) to 437% (daunomycin) in difference between the lowest and highest $LD_{10}$, the change in the number of Fibonacci dose escalation steps that would occur is between zero and one for the majority of these drugs.

## Discussion

Freireich et al. (1966) compared toxicity data for cancer chemotherapeutic agents in animals and humans and showed that there is a relationship for specific drugs between the $LD_{10}$ of mice, rats, hamsters, dogs, and monkeys and the human MTD when doses are expressed on the basis of surface area. Homan et al. (1972) calculated the probability of exceeding the human MTD from drug doses calculated as fractions of the MTD in the dog, monkey, or the more sensitive of these two species. When the initial human dose is one-third of the MTD ($mg/m^2$) in the more sensitive species, the probability of exceeding the MTD in humans is

about 6%; when either the dog or the monkey is used alone, this probability is about 10%. Goldsmith et al. (1975) analyzed mouse, dog, and monkey toxicity data in relation to dose schedules for a number of older anticancer agents, and indicated that the mouse was a valuable predictor of quantitative toxicity in humans for these drugs. A drawback in their analysis, however, was the variable clinical methodology used in arriving at the MTD, and the necessity for wide extrapolation from noncorresponding dose schedules to compare human with animal data.

Our study analyzes the consequences of using one-third of the large-animal TDL as a starting dose in humans, and compares it with the utilization of mouse data for this purpose. The number of Fibonacci dose escalation steps required to reach a human MTD for subsequent Phase II trials is often excessive when the large-animal data are used (Table 4): eight of twelve drugs would have required six or more dose escalation steps to reach a human MTD. When the initial starting dose selected for Phase I trials is too low, it follows that a large number of dose escalation steps are required to reach a human MTD. This contributes to time-consuming Phase I trials and to considerable delay in initiating efficacy trials with new agents. This delay may lead, in addition, to frequent deviations from the initial plan of investigation. On the other hand, if one-third of the mouse $LD_{10}$ were the initial starting dose, ten of eleven drugs would have required less than six dose escalation steps to reach a human MTD.

Looking at Table 4 from another point of view, none of the 12 drugs would have required less than one dose escalation step to reach a human MTD if the initial starting dose had been chosen from the large-animal TDL. Selection of an initial starting dose in humans from one-third of the mouse $LD_{10}$ would generally have been adequate, although for a given schedule of anguidine and piperazinedione, the dose-limiting toxicity might have been encountered on the first step. With 3-deazauridine, an initial starting dose selected from one-third of the mouse $LD_{10}$ would have resulted in more dose escalation steps than were needed with the initial starting dose selected from large animal data.

To put these quantitative considerations in perspective, it should be emphasized that monitoring of toxicity in patients is the best guide to dose escalation and delineation of subclinical and clinical toxicities. This monitoring, coupled with expert clinical support of potential complications, adds immeasurably to the safety of trials even if toxicity is encountered in the first or second dose escalation steps.

One must also be aware that it is not unusual for mouse $LD_{10}$ data to show wide variations when obtained from a number of different experimental conditions (Guarino et al., 1979). Differences of up to several hundred percent may be recorded, presumably as a result of different mouse strains, route of drug administrations, and drug vehicles being used. When one analyzes the impact of such variability of the number of Fibonacci dose escalation steps that would occur if the mouse $LD_{10}$ were used as a basis for selecting the initial starting dose in humans, no substantial changes are encountered.

From this review, mice appear to be useful quantitative predictors for selecting initial starting doses in humans. Moreover, mice offer great potential in terms of economy, numbers for statistically valid results, correlation of toxicity in normal versus tumor-bearing animals, schedule dependency, and dose-response toxicologic curves. Utilization of toxicity data in mice for clinical trials should be further encouraged.

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