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# The role of comparative pharmacokinetics in the planning of human dose escalation: the experience with diacetyldianhydrogalactitol*

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**Summary.** The pharmacokinetics of diacetyldianhydrogalactitol (DADAG) was compared in mice, rats, and humans. The ratios of human therapeutic dose (ThD) to the $LD_{10}$ were 8 and 5 in mice and rats, respectively. The ratios of the corresponding AUCs of DADAG were 20 and 17, whereas those of dianhydrogalactitol (DAG), the main, active metabolite of DADAG, were 8 in both species. The lower human-to-rodent ratio for DAG was due to the fact that twice as much DAG was formed in the animals. Other factors contributing to the larger AUC in man were the 3–5 times smaller distribution volume found in humans as well as the lower hexitol sensitivity of human bone marrow cells. We conclude that in addition to the distance between the AUCs of the $LD_{10}$ and of the human starting dose, interspecies pharmacokinetic differences should also be considered in planning the rate of dose escalation.

## Introduction

Diacetyldianhydrogalactitol (DADAG) is a new alkylating hexitol derivative which is presently under phase II clinical evaluation in Hungary [4, 9]. The starting dose, 15 mg/$m^2$, was calculated as the mean of 1/3 of the highest non-toxic doses in rats and dogs. The dose recommended for therapeutic trial was 900 mg/$m^2$ given every 4th week. This level was reached after 12 steps according to the modified Fibonacci dose escalation plan and was about eight times higher than the $LD_{50}$ in mice, 110 mg/$m^2$ [4]. To reduce the number of subjects receiving clinically irrelevant, small doses, Collins et al. [1] have recently recommended that the dose escalation strategy be based on the relation of the AUCs measured in mice and humans following the administration of the $LD_{10}$ and the starting human dose, which should be calculated as 1/10 of the $LD_{10}$ in mice. This retrospective, comparative evaluation of the pharmacokinetics of DADAG in mice, rats, and humans was undertaken to further evaluate the pharmacokinetic parameters that should be considered in the extrapolation of preclinical data to clinical drug trials.

## Materials and methods

DADAG was kindly provided by the Chinoin Pharmaceutical and Chemical Works. The drug was dissolved in saline immediately before use.

Groups of four male Wistar H Riop rats were anesthetized with i.p. injection of 12.5 mg/kg urethan. Polyethylene cannulas were inserted into the common carotid artery and external jugular vein. The former was used for blood collection, whereas the latter served as the injection port. During the experiment the rats were placed on a heated plate. Heating was regulated by a thermocouple placed into the rectum to maintain the body temperature between 36°–38° C. Injections of 90, 150 ($LD_{10}$), and 180 mg/$m^2$ DADAG dissolved in physiological saline were given over 1 min i.v. or i.p. Blood samples (0.2 ml) were collected before and 1, 2, 3, 5, 10, 20, 40, 60, 80, 100, and 120 min after drug inoculation.

(BALB/c × CBA)$F_1$ female mice were injected i.p. with 97.5 mg/$m^2$ ($LD_{10}$) DADAG. The mice were sacrificed at 3, 5, 7, 10, 13, 16, 20, 30, 40, 60, 80, and 100 min after drug administration. Each time point was calculated as the mean value of five animals.

The blood specimens were drawn into heparinized PP microtubes and centrifuged and the plasma was promptly frozen. The parent drug and the active metabolite, dianhydrogalactitol (DAG), the latter as *n*-butylboronate, were assayed by gas-liquid chromatography (GLC) using FID (CHROM 42 gaschromatograph with Digint 34 μ integrator) essentially as previously described [3]. Plasma samples of 100 μl were extracted with 1.5 ml ethylacetate in the presence of 0.5 g $MgSO_4$ sic. The organic layer was evaporated under $N_2$. *n*-Hexadecane was used as the internal standard. The glass column (1220 mm × 3 mm), packed with 100/120 CW-H.P/5% SE-30 (Applied Sci, Oud-Beijerand, Nl), was used under the following conditions:

column temperature 130° C, injector and detector emperature 150°, carrier gas ($N_2$) flow rate 25 ml/min
$RRT_{DADAG} = 0.56$, $RRT_{DAG} = 0.75$

The plasma concentration curve of DADAC following i.v. inoculation was fitted with the equation

$$Ci(t) = A_1\exp(-\lambda_1 t) + A_2\exp(-\lambda_2 t). \quad (1)$$

The equation for fitting DAG points was derived from a three-compartment open model as a complex system

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considering DAG as a compartment of DADAG kinetics, where $T_i = 0.693/\lambda_i$ and $\tau$ = lag time [3]:

$$C_i(t) = A_1\exp[-\lambda_1(t-\tau)] + A_2\exp[-\lambda_2(t-\tau)] - -(A_1+A_2)\exp[-\lambda_3(t-\tau)]. \quad (2)$$

In the treatment of the plasma data after i.p. injection, the extremely rapid absorption phase was ignored. In these cases the fittings of the DADAG and DAG kinetics were done independently. The equation used for DADAG experiments had the following form:

$$C_i(t) = A_1\exp(-\lambda_1 t) + A_2\exp(-\lambda_2 t). \quad (3)$$

In the rat only one elimination phase was observed; therefore, the equation used was

$$C_i(t) = A\exp(-\lambda t). \quad (4)$$

The curves for DAG in both species were fitted as

$$C_i(t) = A_1\exp(-\lambda_1 t) + A_2\exp(-\lambda_2 t). \quad (5)$$

The apparent volumes of distribution for DADAG in the central compartment ($V_c$) and in the terminal phase ($V\lambda_2$) were calculated by dividing the dose in mg/kg body weight in the first case by the apparent initial plasma concentrations ($A_1 + A_2$) and the latter case by the product of AUC and $\lambda_2$. The volume of distribution at steady state ($V_{ss}$) was calculated from $V_{ss} = Cl.\bar{t}$, where Cl is the clearance and t is the mean residence time. The human data were taken from our earlier reports [3, 4].

The drug sensitivity of the granulocyte-macrophage colony-forming cells in culture (GM-CFUc) were assayed on bone marrow cells of (BALB/cxCBA)$F_1$ mice and of patients who underwent diagnostic biopsy. The cells were incubated at 37° C for 60 min. Surviving cells were estimated in soft agar culture as previously described [5, 6, 11].

$D_o$ was calculated as the drug concentration reducing the number of surviving GM-CFUc to $e^{-1} \sim 0.37$ at the exponential portion of the dose-surviving curve.

## Results

The acute toxicity data for DADAG and DAG are summarized in Table 1. The plasma concentration time curve of rats following the i.v. inoculation of DADAG had two linear phases, as does that of man [3], except that the slopes were much steeper in rats (Figs. 1 and 2a). The half-lives of the distribution and elimination phases varied between 0.69–2.75 min and 63–148 min, respectively. The corresponding values in humans ranged from 8 to 38 and 1200 to 5940 min (Table 2). The maximum concentration of the main metabolite, DAG, was reached in 5–10 min in rats

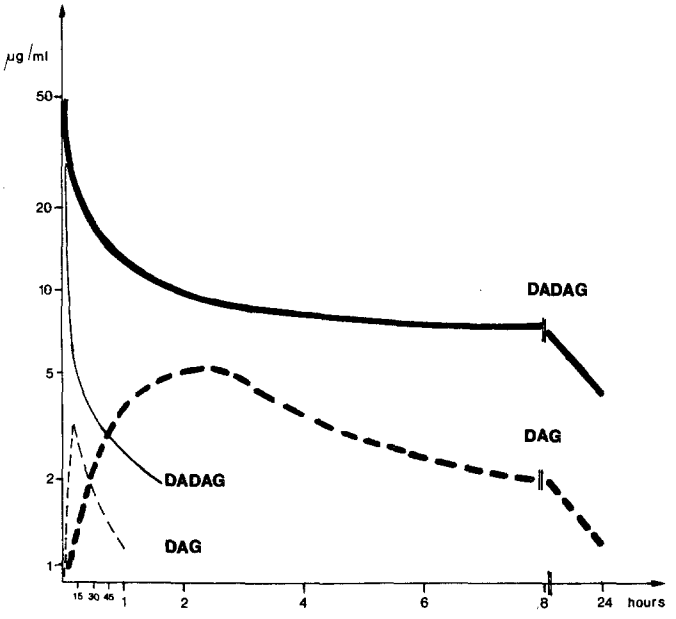


**Fig. 1.** Plasma level curves of DADAG and DAG in humans and rats after the i.v. inoculation of the ThD and the $LD_{10}$, respectively. ——— DADAG human; ----- DAG human; ——— DADAG rats; ----- DAG rats

**Table 1.** Acute toxicity values for DADAG and DAG

| Animal (male) | Route of administration | DADAG | | | DAG | | | DAG/DADAG μmol |
|---|---|---|---|---|---|---|---|---|
| | | mg/kg | mg/m² | μmol/kg | mg/kg | mg/m² | μmol/kg | |
| $LD_{50}$ | | | | | | | | |
| Mouse (Swiss) | i.p. | 34[a] (30.5–37.5) | 102 | 149 | 14.3[b] (13.8–14.8) | 42.9 | 98 | 0.66 |
| | i.v. | 39 | 117 | 170 | 20.5 | 61.5 | 140 | 0.82 |
| Rat (Wistar) | i.p. | 28 | 168 | 122 | 11.2[c] (10.2–12.3) | 67.2 | 76 | 0.62 |
| | i.v. | 36 | 210 | 152 | 16–18[c] | | 116 | 0.76 |
| $LD_{10}$ | | | | | | | | |
| Mouse | i.p. | 29.8–32.5 | 97.5 | 130 | 11.9 (10.2–12.3) | 35.7 | 82 | 0.59 |
| | i.v. | 36 | 108 | 157 | 18.0 | 54 | 123 | 0.78 |
| Rat | i.p. | 25.5 | 153 | 111 | 9 | 54 | 62 | 0.56 |
| | i.v. | 25 | 150 | 109 | | | | |

[a] Data from [9]
[b] Data from [7, 8]
[c] Data from [7]

**Table 2.** Pharmacokinetic parameters of DADAG for mice, rats, and humans

| | Mice | Rats | | | | Humans |
|---|---|---|---|---|---|---|
| Dose mg/m² (mg/kg) | 97.5 (32.5) | 90 (15) | 150 (25.0) | 180 (30.0) | 180 (30.0) | 900 |
| Route of administration | i.p. | i.v. | i.v. | i.v. | i.p. | i.v. |
| $A_1$ µg/ml | 44.8 | 20.0 | 50.5 | 50.0 | – | 39.9 ±13.4 |
| $A_2$ µg/ml | 2.60 | 2.89 | 3.51 | 6.57 | 5.70 | 11.5 ± 2.9 |
| $\lambda_1$ $min^{-1}$ | 0.252 | 1.0 | 0.32 | 1.50 | – | 0.051± 0.031 |
| $\lambda_2$ $min^{-1}$ | 0.010 | 0.011 | 0.006 | 0.008 | 0.012 | $5.33 \times 10^{-4} \pm 3.17 \times 10^{-4}$ |
| $T_1$ min | 2.75 | 0.69 | 2.13 | 1.5 | – | 13.58 ±22.35 |
| $T_2$ h | 1.15 | 1.05 | 2.46 | 1.5 | 0.98 | 21.67 ±36.43 |
| $\bar{t}$ h | 1.5 | 1.4 | 2.2 | 2.0 | 1.39 | 48.1 ±50.5 |
| peak concentration of DAG, µg/ml | 7.8 | 2.2 | 3.2 | 5.4 | 2.9 | 3.6 ± 1.9 |
| time of peak concentration of DAG, min | 5 | 10 | 10 | 5 | 10 | 84 ±24 |
| τ min | 2.5 | 3.3 | 3.3 | 2.0 | 5 | 24 ± 8 |
| $V_c$ l/kg | 0.69 | 0.66 | 0.46 | 0.53 | 5.26 | 0.36 ± 0.22 |
| $V_{\lambda 2}$ l/kg | 10.31 | 7.37 | 11.26 | 7.89 | 7.57 | 1.98 ± 1.05 |
| $V_{ss}$ l/kg | 4.93 | 3.61 | 4.73 | 4.04 | 4.05 | 1.60 ± 0.22 |
| Cl liter per h/m² | 9.88 | 15.52 | 12.94 | 12.10 | 17.49 | 4.086± 1.476 |

The animal pharmacokinetic values were derived from the mean curve of 4–5 individual experiments. Total number of animals: 60 mice (5 animals for each time point), 16 rats (4 animals in each group)
The human data are the mean ± SD of 18 pharmacokinetic determinations

and in 60–120 min in patients. In both rats and humans its maximum concentration at the $LD_{10}$ and ThD was around 3–4 µg/ml. The terminal segments of the DADAG and DAG curves ran parallel, suggesting that the kinetics of the metabolite are dependent on the rate of formation.

The apparent volume of the central compartment ($V_c$) was of the same size in rats and humans; the volumes of distribution at steady state ($V_{ss}$) and at the terminal phase ($V_{\lambda 2}$), however, were much larger in rats (Table 2). The AUC of DADAG increased proportionally to the doses between 90 and 180 mg/m². The relative size of the AUC value of DAG compared with that of DADAG was considerably greater in rats than in humans (Table 3).

Drug absorption from the peritoneal cavity was rapid in both rats and mice (Figs. 2b and 3). The maximum concentration of DADAG was reached in about 5 min in rats. The absorption rate constant was estimated to be 0.3 $min^{-1}$. The ratio of the AUC measured after i.p. and i.v. inoculation was 0.69. For DAG, the peak concentration after i.p. injection was observed at 10 min and the above-described AUC ratio was 0.65.

In mice, the absorption rate constant of DADAG from the peritoneal cavity was 1 $min^{-1}$. The drug was absorbed so rapidly that the curve could best be fitted by assuming i.v. injection and neglecting the absorption phase. The half-lives of the two phases of the drug disposition were 2.8 and 69 min, respectively. The peak of DAG concentration appeared at 5 min and was greater than in either rats or humans. As in rats, more DAG was formed from DADAG in mice than in man.

With increasing concentration of DAG or DADAG, the number of surviving CFUc decreased exponentially. This suggests that the effect of these drugs on CFUc is not cell-specific. The Do values, which represent the drug con-

**Table 3.** AUC of DADAG and DAG in mice, rats, and humans

| Dose mg/m² | Route of administration | $AUC_{DADAG}$ | | $AUC_{DAG}$ | | $AUC_{DAG}/AUC_{DADAG}$ (µmol h/l) |
|---|---|---|---|---|---|---|
| | | µg h/ml | µmol h/liter | µg h/ml | µmol h/liter | |
| Mice | | | | | | |
| 97.5 | i.p. | 9.87 | 43.3 | 4.85 | 32.2 | 0.77 |
| Rats | | | | | | |
| 90 | i.v. | 5.80 | 25.44 | 3.60 | 24.6 | 0.77 ± 0.14 |
| 150 | i.v. | 11.59 | 50.83 | 4.70 | 32.2 | |
| 180 | i.v. | 14.88 | 65.26 | 7.23 | 49.4 | |
| 180 | i.p. | 10.29 | 45.13 | 4.70 | 32.2 | |
| Humans | | | | | | |
| 900 | i.v. | 195 ± 57 | 848 ± 248 | 37.1 ± 14.0 | 254.10 | 0.32 ± 0.18 |

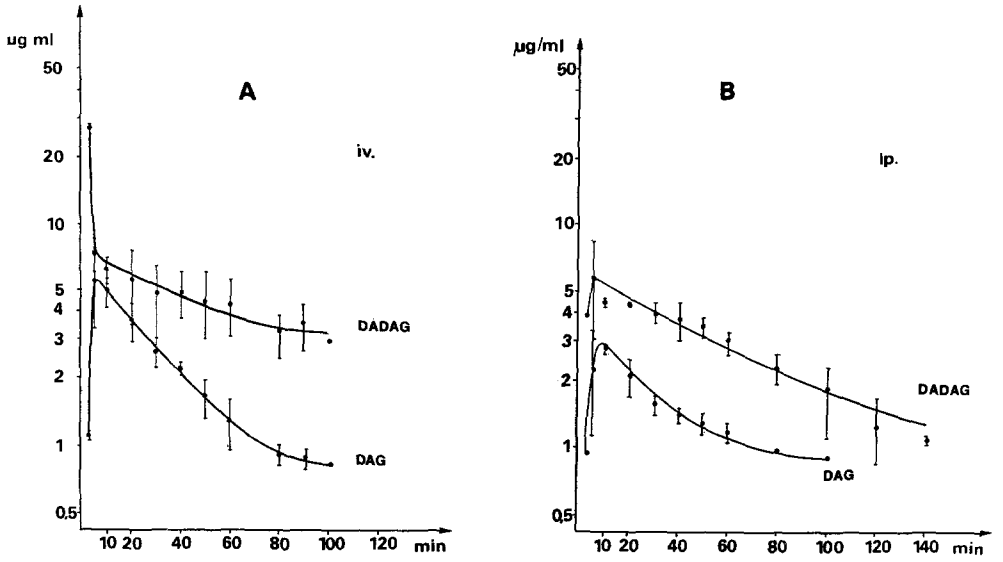


**Fig. 2.** Plasma level curves of DADAG and DAG in rats after the i.v. **(A)** and i.p. **(B)** inoculation of 180 mg/m$^2$ of DADAG. Vertical bars represent SD

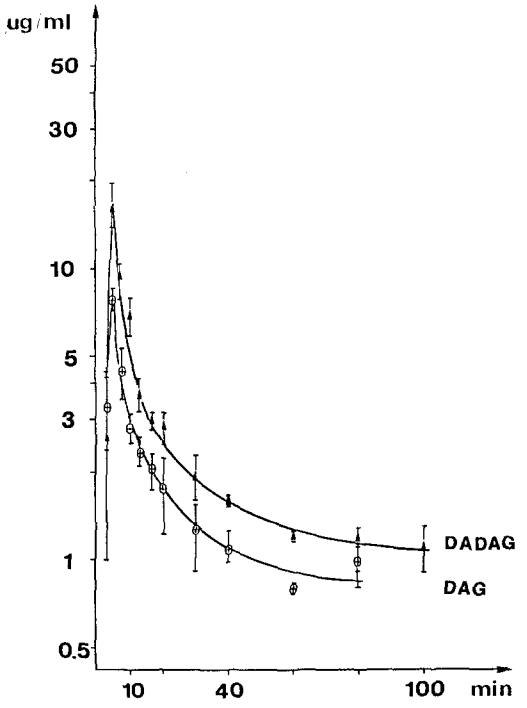


**Fig. 3.** Plasma level curve of DADAG and DAG in mice after the i.p. inoculation of 97.5 mg/m$^2$ of DADAG. Vertical bars represent SD

**Table 4.** Sensitivity of human and murine bone marrow macrophage-granulocyte colony-forming units in cell culture to DADAG and DAG

| | DADAG µmol/l × 1 h | | DAG µmol/l × 1 h | |
|---|---|---|---|---|
| | Human | Mouse | Human | Mouse |
| $D_0$ | 57.3 | 48.3 | 17.2 | 17.8 |
| 90% inhibition | 197.3 | 139.0 | 55.3 | 41.7 |

Incubation at 37° C for 1 h

centration reducing the number of surviving GM-CFUc to $e^{-1} \sim 0.37$ at the exponential portion of the dose-survival curve, were much larger in the case of DADAG, indicating that DAG is the more potent inhibitor of cell proliferation ($P < 0.001$ for both humans and mice) (Table 4). The human and murine dose-survival curves ran parallel as shown by the similar $D_0$ values, but the human curve was shifted to the right. Consequently, the human doses of both DADAG and DAG that caused 90% inhibition of colony formation were larger ($P < 0.001$) [5, 6, 9, 11].

## Discussion

In both mice and rats the lethality curves are very steep. The $LD_{10}$ values are only slightly lower than the $LD_{50}$, which is around 110 mg/m$^2$ in mice and 180 mg/m$^2$ in rats (Table 1). No significant difference was observed between the lethal doses given i.v. or i.p.; however, the therapeutic activity was higher after i.p. treatment [9]. Neither did we find any major deviation in the metabolic pattern or in the distribution and elimination of DADAG given by these two routes to rats. Since the determination of the starting human dose was done on the basis of $LD_{10}$ determined in mice after i.p. and in rats after i.v. injection, the pharmacokinetic parameters determined using these application routes will be used for comparison. Between 90 and 180 mg/m$^2$ the pharmacokinetics of DADAG were linear in the rats. In patients, the linearity could be proven between 15 and 300 mg/m$^2$; the plasma level increase of DADAG later became progressively lower with increasing doses [3]. The dose-AUC curve became so flat that no statistically significant difference could be proven between the AUCs or the clinical effects measured after the inoculation of 690, 915, and 1050 mg/m$^2$ of DADAG. The interindividual variations in AUC at this dose range were greater than those related to the dose escalation. Nevertheless, a significant correlation between the AUC and the clinical effects could readily be established [4]. Considering these peculiar nonlinear pharmacokinetics of DADAG, 900 mg/m$^2$ was recommended as the ThD, which value will be used hereafter. The human pharmaco-

kinetic parameters considered for comparison are the mean values of patients treated with doses ranging from 690 to 1050 mg/m$^2$.

The ratios of the human ThD/$LD_{10}$ were 8 and 5 for mice and rats. The corresponding ratios of the AUCs measured at these doses were even greater, 20 and 17, respectively. Clearly, similar pharmacologic effects appear at quite different AUC values of the parent compound in various species. It is known, however, that DAG, the major metabolite of DADAG, is more potent than the parent compound. Its $LD_{10}$ and ThD levels were 2–3 times lower than those of DADAG on molar bases [2, 4]. The human ThD-to-$LD_{10}$ ratios calculated for the liberated DAG were 8 for both species in the present experiment. The 50% decrease of the AUC ratios is easily explained by the finding that in the rodents the AUC of DAG compared with that of DADAG is double that observed in humans, proving that DAG is the carrier of the pharmacologic effects after DADAG treatment.

There are, however, other interspecies differences which may contribute to the large human-to-animal AUC ratios of both DADAG and DAG. The apparent volume of the central compartment ($V_c$) at ThD in man approximated the total body water. The aparent volumes of distribution at steady state ($V_{ss}$) and at the terminal phase ($V_{\lambda 2}$) were 4.5 and 5.5 times higher. The $V_c$ in patients and in rodents were about the same size, whereas $V_{ss}$ was 2.5–3 times and $V_{\lambda 2}$ was 4–5 times higher in rodents. The relative meaning of these volume terms is confusing, since $V_{\lambda 2}$ is a hybrid parameter and thus reflects changes in elimination as well as distribution [10]. In contrast, $V_{ss}$ is independent of elimination and thus should directly reflect alterations in distribution processes. As a result, the present data suggest that DADAG may have different distribution processes (tissue binding) in addition to the different rates of elimination (metabolism) in rodents and humans. The magnitude of $V_{ss}$ in patients, and especially in rodents, suggests that tissue binding plays an important role. Although we do not know the amount of drug used de facto for the alkylation of DNA, we may assume that the cytostatic effect is related to the amount of drug reaching the peripheral compartment, i.e., the tissues.

Finally, the target cells themselves might react differently to the drugs. In the case of hexitols, the bone marrow cells of mice seem to be slightly more sensitive than those of humans. The human-to-mouse ratios of the CxT products causing a 90% decrease of the surviving fraction of GM-CFUc were 1.4 and 1.3 for DADAG and DAG, respectively. It is notable that in man the AUCs for both compounds are much larger than the CxT causing 90% cell kill in vitro. In mice the opposite is true, suggesting that beyond the bone marrow toxicity, which is the dose-limiting side effect, other factors, i.e., gastrointestinal lesions, might contribute to the lethality in animals. In our opinion, only all the above-described dissimilarities together can adequately account for the large differences in AUC observed at equitoxic doses in the various species.

The present example does not refute the proposal of Collins and associates [1] to tailor the dose escalation program according to the distance between the AUC measured after the inoculation of the $LD_{10}$ and that measured after the human starting dose; it should serve much more as a warning against attaching exclusive importance to the AUC. It is recommended that all the pharmacokinetic parameters showing significant deviations from the animal data in the first human experiments should be jointly considered in the planning of the phase I trial. If used with precaution, the pharmacokinetic results may give substantial support for the safe, rapid, and economic evaluation of new cytostatic agents in patients.

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