A Multivariate Analysis of Ca-DTPA-Effectiveness in Removing ^{241}Am from the Rat

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^{241}Am, Ca-DTPA, rat

The dependence on time of the dose-effect relationship was studied for the removal of ^{241}Am from the skeleton and liver of the rat by Ca-DTPA. Due to the linearity of the dose-effect-curves (in a log-log scale) as well as to the linear dependence of the slope on the logarithm of time, simple equations were derived which describe the mobilization of ^{241}Am as influenced by DTPA-dosage and time of treatment.

In general, the effectiveness of chelating agents in removing internally deposited radionuclides from the mammalian body depends markedly on the chelate dosage as well as on the time of treatment[1]. This holds also for the mobilization of ^{241}Am by DTPA (diethylenetriaminepentaacetate)[2–5]. In this paper, which deals with the retention of ^{241}Am in the rat, an attempt is made to quantitize the influence of the factors mentioned above. Such an analysis of the effectiveness pattern should not only be the basis of understanding the action of multiple chelate doses but might also yield information about the metabolic behaviour of ^{241}Am.

Methods

Female albino rats of the Heiligenberg-strain were intravenously injected with ^{241}Am-citrate (0.3 μCi in 0.25 ml; pH 7.5-8.5; prepared according to[6]) and received a single intraperitoneal injection of Na_3-[Ca-DTPA] at different times after ^{241}Am; the chelate dosage ranged from 0.03-1.0 mmole · kg^{-1}. For reasons discussed elsewhere[5], the rats were sacrificed 7 days after administration of DTPA, excepted for those treated on the 64. day, which were killed 12 days after treatment. Details relating to the assay of α-activity of the organs by liquid scintillation counting are described elsewhere[7]. The ^{241}Am-content of the skeleton was calculated by multiplying by 20 the activity of one femur. In order to describe the chelate efficacy, the ^{241}Am-content of liver and skeleton is presented as percentage of the corresponding control. Parts of the data were calculated from values presented earlier[5].

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Results and Discussion

The dependence of chelate effectiveness on dosage as well as on the time of its administration is shown in Figs 1 and 2. The dose-effect curves were calculated by means of regression analysis and found to be linear in a log-log scale. Consequently, the dose-dependence can be described by the equation.

$$\log y = a + b \log x$$

where x denotes the dosage [μmol · kg^{-1}] and y the ^{241}Am-content of the organ expressed as a percentage of the control. In the case of the liver, one experimental point, *i. e.* 1 mmole · kg^{-1}, injected after 1.5 min, was omitted from the calculation, since the effect of this dose is distinctly lower than the corresponding extrapolated value of the linear regression line (Fig. 2).

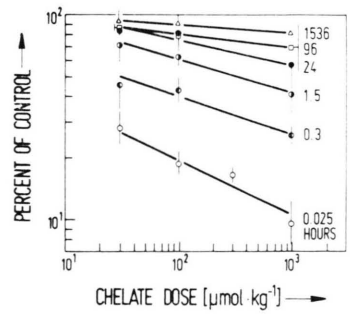

Fig. 1. Influence of chelate dosage and time interval between ^{241}Am-injection and treatment (figures on the right side) on the effectiveness of Ca-DTPA in removing ^{241}Am from the skeleton. Geometric means of 5 rats on the average; for the sake of clarity, one-tailed fiducial limits in some cases (P = 0.05). Curves were calculated by variance analysis.

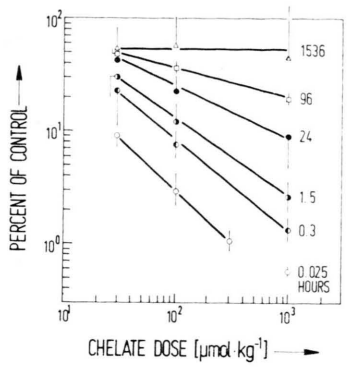

Fig. 2. Influence of chelate dosage and time interval between ^{241}Am-injection and treatment (figures on the right side) on the effectiveness of Ca-DTPA in removing ^{241}Am from the liver. Geometric means of 5 rats on the average; for the sake of clarity, one-tailed fiducial limits in some cases (P = 0.05). Curves were calculated by variance analysis.

As can be seen from Fig. 3, there is a linear dependence of the slope b on log t [hours], whereas the parameter a shows a curvilinear dependence. The regression of both parameters on time was calculated and, by substitution in the above equation, the following expressions were obtained for skeleton:

$\log y = [2.037 + 0.089 \log t - 0.031 (\log t)^2] - [0.172 - 0.045 \log t] \log x$

and liver:

$\log y = [2.467 - 0.036 \log t - 0.057 (\log t)^2] - [0.678 - 0.193 \log t] \log x$

By use of these equations it is possible to calculate dose-effect-functions for any given time as well as the time dependence of effectiveness for any given dose; the latter calculation is given in Fig. 4 and shows good fit between the experimental points and

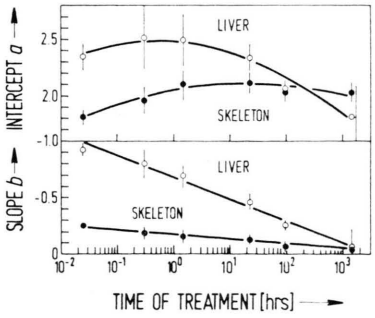

Fig. 3. Dependence on time of the parameters a and b of the dose-effect curves given in Figs 1 and 2. The vertical bars are fiducial limits (P = 0.05). For further details see text.

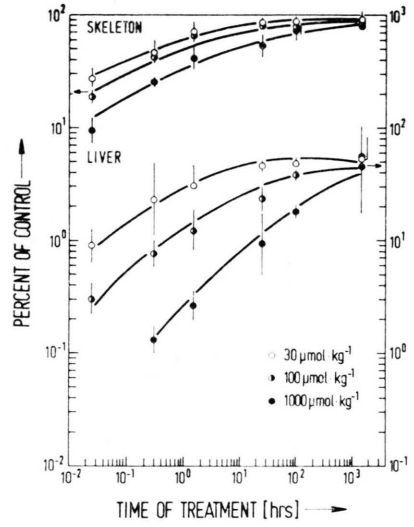

Fig. 4. Retention of ^{241}Am in skeleton and liver of the rat as dependent on the time interval between injection of ^{241}Am and Ca-DTPA. Geometric means of 5 rats on the average; for the sake of clarity, one- tailed fiducial limits in some cases (P = 0.05). Functions calculated with the equations given in the text.

the calculated regression line. Nevertheless, the mainly formal nature of the equations should not be overlooked; one should be cautious with extrapolation beyond the actually investigated limits of dosage and time.

A marked deviation from linearity, for example, is already evident in the case of the liver and the highest DTPA-dose, given after 1.5 min (Fig. 2). However, the efficacy of lower doses (< 30 μmoles · kg^{-1}) is more interesting from the practical point of view. In order to elucidate this question, an additional experiment was performed: A DTPA-dose of [10 μmoles · kg^{-1} was administered after 1.5 min or 24 hours. The ^{241}Am-burden of the liver amounted to 24.7 and 78.0 %, respectively, of the control; that of the skeleton was 34.2 and 95.1 % respectively, of the control. These values compare quite well with those of the extrapolated regression lines in Figs 1 and 2. Thus, an extrapolation up to 10 μmoles · kg^{-1} seems to be justified and feasible, at least for relatively early times of treatment.

In our study, the decrease of chelate efficacy with increasing time interval was found to be paralleled by a corresponding decrease of the slope of the dose-effect-curves A common underlying mechanism may be assumed. Generally, the time-dependent loss of chelate efficacy suggests two possibilities: 1. The

fraction of ^{241}Am, which — due to its deposition site and/or its chemical reactivity — becomes unavailable to DTPA, increases with time. 2. ^{241}Am is retained from the very beginning by at least two compartments which are characterized by different binding affinities to ^{241}Am and, thus, by a different residence time of ^{241}Am as well as different response to DTPA; with increasing time, the loosely bound fraction becomes depleted due to spontaneous excretion, and the effect of DTPA becomes limited because of the prevalence of the tightly bound fraction. It can be easily verified numerically that the existence of a multicompartment system as outlined above will lead to a decrease of the slope of the dose-effect curves with increasing time.

Taking into account that the skeletal burden of ^{241}Am remains virtually constant over long periods of time, we are obviously dealing with situation 1. in this case, *i. e.*, in terms of the compartment analysis, with a so-called catenary system. The response of the liver, from which spontaneous elimination is a rather fast process in the rat[4, 8, 9], is, shortly speaking, compatible with both assumptions. However, the repeatedly mentioned fact that the influence of the highest dose, administered after 1.5 min, is distinctly lower than should be expected on the basis of the linear dose-effect relationship for doses between 10 and 300 μmoles · kg^{-1} (Fig. 2) may be taken as evidence in favour of the situation 2., *i. e.*, of a so-called mammillary compartment system. The assumption of more than two ^{241}Am-compartments in bone and liver is corroborated by autoradiographic and biochemical findings[10–13].

The behaviour of the intercept *a* (Fig. 3) suggests that — in addition to the heterogeneity of the binding sites mentioned above — two basically different phases of DTPA-efficacy are involved. Indeed, it has been shown[14] that after intravenous injection of ^{241}Am-citrate there is a significant amount of activity in the blood plasma only up to about 90 min. Consequently, during this time interval interaction of DTPA with ^{241}Am takes place mainly in the plasma, *i. e.*, we are dealing with a prevention of ^{241}Am-deposition, whereas after about 90 min a genuine mobilization from the organs dominates.

It has been pointed out by TREGUBENKO *et al.*[15] that the use of chelating agents might yield some information about the metabolic behaviour of radionuclides in the body, not accessible to biochemical and/or histological methods. Although the functions derived from the present study reflect changes of the binding of ^{241}Am by different endogenous ligands, it is not yet possible — due to the complex nature of these functions and, in particular, the lack of additional data — to correlate our findings with concrete physiological and biochemical processes.

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