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Studies on diphosphonate kinetics

Part I: Evaluation of plasma elimination curves during 24 h

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Abstract. To improve the understanding of diphosphonate affinity to metabolically active bone, the underlying diphosphonate kinetics have been evaluated and compared to Cr-EDTA kinetics. MDP binds to plasma proteins, varying from 25% initially to approximately 70% after 24 h. The renal clearance of diphosphonate is found to be equal to Cr-EDTA clearance. Using simultaneous bolus injection of ^{99}Tc-MDP and ^{51}Cr-EDTA, it has been possible to obtain a coarse estimate of bone uptake of MDP. This uptake is found to correlate well with s-alkaline phosphatase, but since MDP binding to bone is reversible, the plasma elimination curve is not monoexponential. Therefore it has not been possible to describe the uptake of MDP in bone mathematically.

Key words: Bone formation – Chrom – EDTA – Diphosphonates – Protein binding – Renal clearance

Diphosphonates are of great importance in the diagnosis of both focal and generalized bone disease, due to their high affinity to metabolically active bone (Fogelman et al. 1982). Interest in the kinetic properties of diphosphonates has therefore been increasing, starting with the comprehensive studies of Subramanian and coworkers (Subramanian et al. 1972; Subramanian et al. 1975). The main topic of these and following studies was to compare the different diphosphonates in order to improve the quality of skeletal imaging (Todd Makler and Charkes 1980; Castronovo 1980; Knop et al. 1981; Mele et al. 1983). Kinetic studies have, however, also proven useful for understanding of the quantitative measurements of 24 h whole body retention of diphosphonate (WBR) (Fogelman et al. 1978; Hyldstrup et al. 1984), introduced as an index of total skeletal turnover.

Recent reports dealing with diphosphonate kinetics have so far been based on compartmental analysis, using more or less a preconceived compartmental model (Makler and Charkes 1980; Mele et al. 1983). The aims of the present study have been to obtain a noncompartmental description of tracer movements in order to evaluate diphosphonate uptake in bone as well as its renal elimination.

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Materials and methods

A noncompartmental analysis of ^{99}Tc-methylene diphosphonate (Tc-MDP) kinetics was planned. To allow this, ^{51}Cr-EDTA was selected as a cotracer. For the analysis of the plasma elimination, several factors had to be studied: the binding of Tc-MDP to plasma proteins and erythrocytes, the reversibility of the tracer binding to bone, and the renal handling of Tc-MDP. For these purposes, the following experiments were planned:

Experiment 1. The degree of protein binding and its time dependency was studied in five volunteers over 24 h. The plasma proteins were precipitated with trichloroacetic acid (0.306 mol/l), 1 ml serum + 9 ml trichloroacetic acid. To avoid overestimation of the precipitated fraction due to attachment of unbound Tc-MDP to the fluffy precipitate, it was thereafter washed three times with trichloroacetic acid before the counting procedure. This precipitation was also used in the following experiments 3–5.

Experiment 2. In two patients the binding of Tc-MDP to erythrocytes was measured by counting erythrocyte activity after centrifugation and separation of the plasma.

Experiment 3. The renal extraction efficacy of Tc-MDP was determined and compared to that of Cr-EDTA in five patients undergoing catherization of a renal vein due to suspected renal hypertension. With catheters in both the renal vein and the aorta, the a–v difference over the kidney was measurable. Cr-EDTA was chosen as a reference since it is a well established measure of glomerular filtration rate.

Experiment 4. In order to secure parallel slopes in the arterial and venous samples, four volunteers received an intravenous bolus of Tc-MDP and Cr-EDTA and both arterial and venous samples were drawn repeatedly for 6 h.

Experiment 5. Finally, the 24 h plasma curves were determined in five other volunteers, in this study Cr-EDTA was injected simultaneously as a cotracer to serve as a reference to the diphosphonate.

The kits used for all studies were Medronate-II® (Amersham Int. Ltd.) and Krom(^{51}Cr)EDTA® (Amersham Int. Ltd.). The dosage of ^{99m}Tc was 2.0 mCi, resulting in a total count rate in the initial samples of approximately

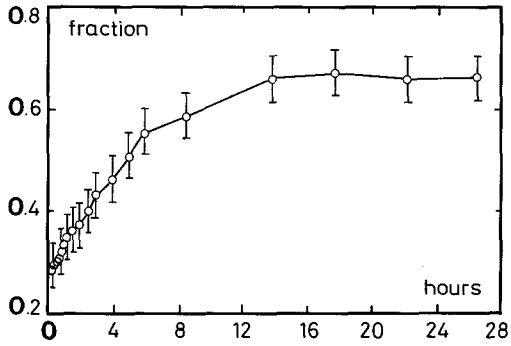

Fig. 1. Time dependency of protein binding of ^{99m}Tc-MDP in the five subjects studied. The binding is given as the fraction between the precipitated activity and the total activity in the plasma samples (mean $\pm$ 1 SD)

Table 1. Erythrocyte binding of ^{99m}Tc-MDP in two subjects during 24 h, given as fraction of the whole blood activity

Patient no.	Erythrocyte binding		
	at 1 h	at 3 h	at 24 h
1	0.035	0.049	0.144
2	0.038	0.050	0.129

10^5 cpm/ml. For ^{51}Cr the dosage was 150 uCi, giving approximately 10^4 cpm/ml. The methylene diphosphonate dose was 5.0 mg. For the first 6 h both arterial and venous samples were drawn, but for the remaining 18 h only venous samples were obtained. A well type gamma scintillation counter (LKB Wallac, Compugamma®) was used for counting. In all cases the Tc-MDP was counted immediately after the sample period, while the Cr-EDTA was counted at least two days later, corresponding to 8 half lives of ^{99m}Tc.

In accordance with the Helsinki II declaration, all subjects received oral and written information and informed consent was required.

For statistical evaluation, linear correlation analysis, students' t-test for paired observations and curve fitting by least squares was used.

Results

Experiment 1. An example of the protein binding ratio in the five patients is shown in Fig. 1. The calculated slopes are based upon measurements during the first 24 h and are therefore only valid within this interval. The best curve fit for the binding ratio (y) was obtained by the equation:

$y = A_0 - A_1 e^{-B_1 t}$; time t in h.

The mean ($\pm$SD) values were: $A_0 = 0.665$ (± 0.067), $A_1 = 0.388$ (± 0.011), and $B_1 = 0.303$ (± 0.056).

Experiment 2. The ratio of erythrocyte binding in the two subjects is given in Table 1.

Experiment 3. The extraction efficiency of Tc-MDP in the kidney was slightly lower than that of Cr-EDTA, but the difference was not significant (0.174 vs 0.167, $P > 0.2$). The mean ratio between the two efficiencies was 0.95, data for all the investigated subjects are given in Table 2.

Table 2. Renal extraction efficiencies of Cr-EDTA and Tc-MDP in five patients. Etc/Ecr is the ratio between the extraction of the two tracers

Patient no.	Extraction fraction for Cr-EDTA	Extraction fraction for Tc-MDP	Etc/Ecr ratio
1	0.107	0.099	0.95
2	0.214	0.200	0.94
3	0.102	0.086	0.81
4	0.175	0.202	1.15
5	0.273	0.246	0.90
mean	0.174	0.167	0.95
SD	0.073	0.070	0.12

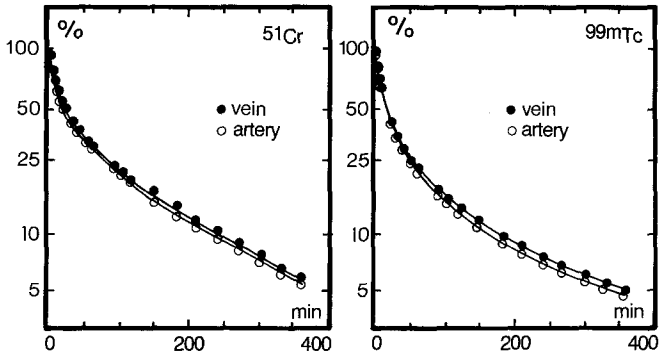

Fig. 2. Comparison of arterial (o) and venous (•) activities after bolus injections of ^{51}Cr-EDTA (*left curves*) and ^{99m}Tc-MDP (*right curves*)

Experiment 4. Differences in arterial and venous elimination curves of Tc-MDP and Cr-EDTA revealed a similar pattern in all four volunteers: initially the arterial level was slightly higher than the venous, but after 15 min–25 min the venous curve intersects and remains higher throughout the study. This is illustrated in Fig. 2, showing one of the studies, which is typical of all four and indicates that the curves for Tc-MDP do not become monoexponential within 6 h, in contrast to the Cr-EDTA-curves.

Experiment 5. This led to the prolonged study, during which plasma elimination curves were digitized for 24 h. The initial 6 h revealed a pattern identical to that of experiment 4, therefore in Fig. 3, for the sake of clarity, only the venous data for Tc-MDP and Cr-EDTA are shown. The curves have been corrected for differences in dose so that the Tc-MDP and Cr-EDTA curves are visually comparable. It is evident that even in the prolonged study the Tc-MDP curves do not reach monoexponentiality and the curves therefore intersect. In each case the curve is supplied with data on renal function (standard Cr-EDTA clearance) and on bone turnover (serum alkaline phosphatase). Comparison of the maximal difference in % of the 1 min value of the two curves (reflecting the part of the Tc-MDP elimination deriving from bone uptake) reveals a positive and significant correlation with s-alkaline phosphatase as demonstrated in Fig. 4 ($r = 0.93$, $P < 0.02$).

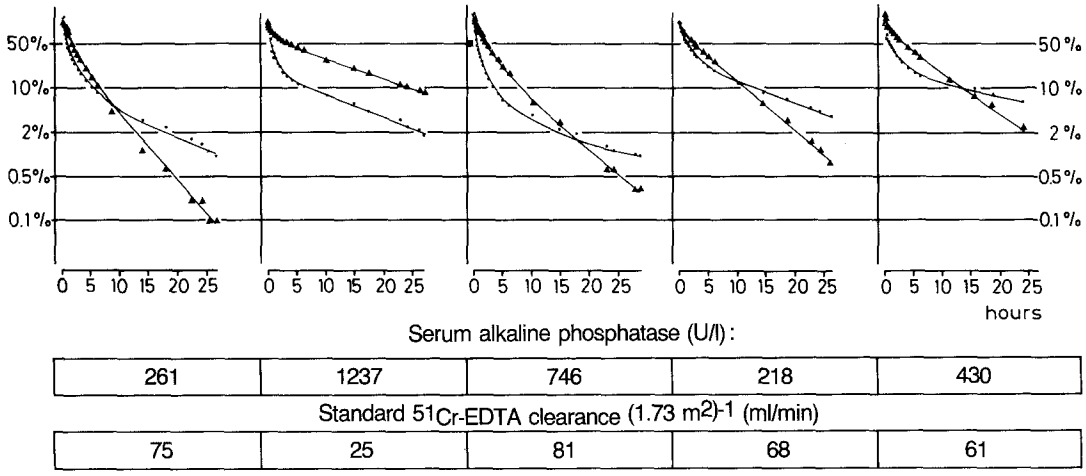

Fig. 3. Measured activity of ^{51}Cr-EDTA (▲) and ^{99m}Tc-MDP (●) in plasma during 24 h. In the five subjects the different levels of bone turnover and renal function are indicated by s-alkaline phosphatase and Cr-EDTA-clearance, respectively. All activities are recalculated to % of measured activity at 1 min. Clinical diagnosis (*left to right*): arterial hypertension, primary hyperparathyroidism, osteomalacia, cerebral haemorrhage, and diabetes mellitus

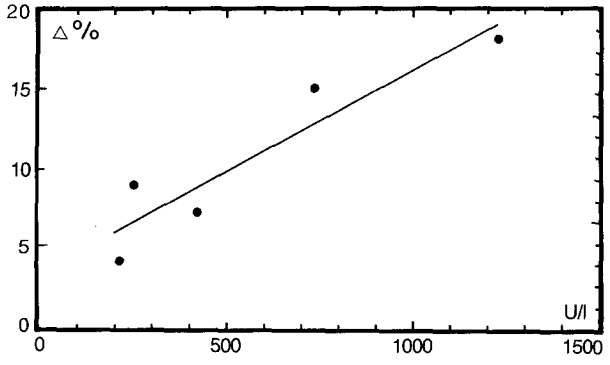

Fig. 4. Correlation between the difference in the % activity of Cr-EDTA and Tc-MDP present at 5 h and the serum alkaline phosphatase in the five subjects in experiment 5, Fig. 3

Discussion

The main purpose of this investigation was to obtain a quantitative description of bone uptake of diphosphonate, which would be of value for the further understanding of the 24 h whole body retention of diphosphonate, a technique which is used in the diagnosis of metabolic bone disease. The planned mathematical description, based on the Henriques-Hamilton principle, requires a monoexponential slope for the plasma elimination curve. We found, however, that even after 24 h the curve does not become linear in the semilogarithmic plot. The main reason for this might be the binding of tracer to plasma proteins and erythrocytes. As demonstrated, the protein binding is considerable and time dependent, varying from 30%–70% during the first 24 h. This differs somewhat from the results of Subramanian et al. (1975), who found a steeper increase in the binding, rising to 100% after 24 h. The erythrocyte binding was only minor and has therefore not been accounted for in the calculations. Even after correction for protein binding, as demonstrated in Fig. 3, the curve did not become monoexponential. The cause of this bending is probably the return of tracer from bone. Although the rate constant for this tracer movement has been found to be small (Makler and Charkes 1980; Mele et al. 1983), the activity gradient from bone to plasma after a few hours is considerable and may therefore prevent the elimination curve from becoming monoexponential.

Because of the intended comparison of Tc-MDP and Cr-EDTA elimination from plasma, knowledge of the renal extraction efficiencies are of importance, to our knowledge previous studies have not dealt with this. We found that the extraction efficiency of Tc-MDP was slightly lower than that of Cr-EDTA, but the difference was not statistically significant. It is of importance, however, that the elimination of the two tracers is equally affected when renal function is reduced. Another important prerequisite is an identical soft tissue uptake. Because of the demonstrated parallel course of the initial slopes and the synchronous intersection of the arterial and venous curves, we find it likely that no major difference exists regarding soft tissue uptake and release.

Concerning Tc-MDP plasma elimination, all subjects revealed the same pattern: initially the activity level was highest in the arterial sample because of the flux from plasma to soft tissue and bone. However, after a short time the curves intersect due to the return of tracer from soft tissue and for the following 5 h the curves are parallel, but still not monoexponential. For Cr-EDTA, the curve did of course become linear in the semilogarithmic plot, and the a–v intersection took place at the same time as for Tc-MDP. Since the lapse of time before intersection is identical for the two tracers, they seem to have the same initial compartmental distribution apart from the bone uptake of MDP. Based on these results we have found it safe to use only venous sampling in the 24 h studies.

For these studies we chose patients with different levels of renal function and different levels of bone turnover, as judged by the conventionally used parameter of bone metabolism, the serum alkaline phosphatase. Since no difference in renal extraction efficiencies for the two tracers was found, no correction factor was necessary. As shown in Fig. 3 there is (of course) good agreement between the slope of Cr-EDTA and the standard Cr-EDTA clearance, but also the elimination of Tc-MDP is slower when renal function is decreased. When looking at the different levels of bone turnover, as indicated by the varying levels of serum

alkaline phosphatase, the difference in the elimination of the two tracers in the first 5 h, correlates well with the serum alkaline phosphatase. Since the diphosphonate elimination does not become monoexponential, an exact mathematical description of the difference in the two curves is impossible. In the two patients with very high bone turnover (Fig. 3, no. 2 and 3 from left) the Tc-MDP curves tended to intersect later, indicating, that in cases of high turnover, the bone uptake is higher and the release of tracer from bone smaller.

Finally we conclude that due to the release of tracer from bone, a mathematical description of the bone uptake of Tc-MDP is not possible. The study has, however, revealed two features of importance to diphosphonate kinetics: a variation in protein binding with time and a renal excretion comparable to that of Cr-EDTA.

These findings may therefore contribute to other forms of noncompartmental analysis, in which the determination of the final slope is not necessary.

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