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Research Article

21 paraplegic patients with recent traumatic spinal cord injury were orally administered 400 (n = 7) or 1,600 (n = 7) mg/d of disodium dichloromethylene diphosphonate (Cl2MDP) and compared with a placebo group (n = 7) to test the preventive effects of the drug on acute bone loss and osteoclastic resorption. Cl2MDP therapy was initiated at a mean of 17.6 d after the onset of paraplegia. The study lasted at least 6 mo, consisting of a 3.5-mo treatment period, and a variable follow-up period. The effects of Cl2MDP were assessed by blood and urine biochemistry, bone histomorphometry on transiliac samples, photon absorptiometry of the tibia and fibula, and radiomorphometry of the femur. The elevation in serum and urinary calcium and in urine hydroxyproline observed in the placebo group did not appear under treatment. With both doses of Cl2MDP there was no further decrease in the bone mineral content. In the treated groups, a smaller percentage increase in osteoclastic population was also noted when compared with the placebo group, but this difference was not significant. There was no mineralization defect induced by Cl2MDP, as shown by tetracycline double labeling. It thus appears that at doses ranging between 400 and 1,600 mg, given as early as possible, Cl2MDP can prevent or reduce the development of the acute bone loss of paraplegic patients, [...]

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Effects of Disodium Dichloromethylene Diphosphonate on Bone Loss in Paraplegic Patients

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ABSTRACT 21 paraplegic patients with recent traumatic spinal cord injury were orally administered 400 ($n = 7$) or 1,600 ($n = 7$) mg/d of disodium dichloromethylene diphosphonate (Cl₂MDP) and compared with a placebo group ($n = 7$) to test the preventive effects of the drug on acute bone loss and osteoclastic resorption. Cl₂MDP therapy was initiated at a mean of 17.6 d after the onset of paraplegia. The study lasted at least 6 mo, consisting of a 3.5-mo treatment period, and a variable follow-up period. The effects of Cl₂MDP were assessed by blood and urine biochemistry, bone histomorphometry on transiliac samples, photon absorptiometry of the tibia and fibula, and radiomorphometry of the femur. The elevation in serum and urinary calcium and in urine hydroxyproline observed in the placebo group did not appear under treatment. With both doses of Cl₂MDP there was no further decrease in the bone mineral content. In the treated groups, a smaller percentage increase in osteoclastic population was also noted when compared with the placebo group, but this difference was not significant. There was no mineralization defect induced by Cl₂MDP, as shown by tetracycline double labeling. It thus appears that at doses ranging between 400 and 1,600 mg, given as early as possible, Cl₂MDP can prevent or reduce the development of the acute bone loss of paraplegic patients, without adverse side effects, though it does not prevent the development of heterotopic ossification.

INTRODUCTION

The bone loss in spinal cord injury patients constitutes a unique model of acute osteoporosis: the reduction in the amount of trabecular bone averages 33% over 6 mo before a new steady state is reached (1). This results from a marked increase in bone resorption transiently associated with a depression in bone formation. The prevention of such a bone loss should rely on drugs that inhibit osteoclastic resorption, and/or agents preventing reduction in bone formation without adverse side-effects. Among the former group of compounds, disodium dichloromethylene diphosphonate (Cl₂MDP)¹ has been shown as a potent and safe inhibitor of bone resorption, *in vitro* (2) and *in vivo* (4) without inhibition of osteoid mineralization (3, 4). Diphosphonates have indeed been used successfully in animals (5, 6) to prevent the effects of immobilization on bone.

The aims of this study were to determine whether Cl₂MDP could have a preventive effect on the acute bone loss of paraplegic patients, and indirectly to assess its inhibitory effect on osteoclastic resorption in a condition of acute bone loss partly resulting from increased osteoclastic resorption.

METHODS

21 paraplegic patients (17 males and 4 females, mean age: 29 yr, range: 15–54 yr) with a neurological level between

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¹ Abbreviations used in this paper: Cl₂MDP, disodium dichloromethylene diphosphonate; TBD, total bone density; TBV, trabecular bone volume; TIOS, osteoid seam thickness index.

T 1 and T 12, were included in the study with their informed consent.

All patients had sustained complete paraplegia <30 d before inclusion in the study (mean delay: 17.6 d, range: 5–29 d) and remained completely paralyzed throughout the investigation. The study lasted at least 6 mo: 3.5 mo under treatment, and between 2.5 and 7 mo of follow up.

Cl_2MDP was supplied in 200 mg (0.55 mmol) capsules and ingested 2 h after breakfast. The patients were divided into three groups of seven (I, II, and III), according to a randomization code unknown to the experimenters. In group I, all seven patients received a placebo. Group II patients received 400 mg/d of Cl_2MDP (2 capsules) and group III patients 1,600 mg of Cl_2MDP (8 capsules). Complete clinical status was assessed before inclusion in the study. Blood and urine assays were performed before treatment, after 2 wk of treatment, 1 mo, and subsequently every month thereafter.

Blood was drawn for calcium (Ca), phosphate (P), alkaline phosphatase (AP), serum glutamate oxaloacetic transaminase, and serum glutamate pyruvate transaminase measurements, and blood counts. Urine was collected for three consecutive days after 2 d of collagen-free diet. Urine total hydroxyproline, calcium, and phosphate were determined, and mean values were expressed per millimole of urine creatinine per day.

Serum creatinine and creatinine clearance were checked weekly during the first month, then monthly. Fluid intake and urinary volume were measured daily.

The amount of bone was evaluated in both tibia and femur. At the lower extremity of the right tibia, Cameron's photon absorptiometry method (7) modified by Bérard (8) was used. At the midshaft of the right femur, on antero-posterior radiographs, the periosteal (P) and endosteal (E) widths were measured with a Vernier dial caliper (0.05-mm divisions) and the cortical width (C) calculated from the equation: $C = (P - E)/2$. The measurements were performed before treatment, at 3 mo, and for absorptiometry only at 6 mo.

The histomorphometric study was performed before and after treatment on samples obtained by transfixing the iliac bone 2 cm behind the anterior-superior iliac spine and 2 cm below the summit of the right iliac crest, using a trephine of 8 mm i.d. The following parameters were measured microscopically with integrating eyepieces or with an image-analyzing computer according to previously published methods: trabecular bone volume (TBV) (9), total bone density (10), relative osteoid volume, trabecular osteoid surfaces, osteoid thickness index (11), trabecular osteoclastic resorption surfaces, number of osteoclasts/mm² of bone section, and periosteocytic lacunae size (12). 4–12 d before the second biopsy, a double tetracycline labeling (13) was obtained by administration of 600 mg of dimethylchlortetracycline per day for 2 d, and then again for 4 d after a 12-d period without medication. The resulting measurements of calcification rate was made on four unstained 20- μm thick nonconsecutive serial sections.

Statistical methods included analysis of variance and *t* test for paired observations with a statistically significant cut-off level at $P < 0.05$. The mean is quoted $\pm \text{SEM}$ in both the text and the tables.

RESULTS

There was no significant difference in any parameter among the three groups before treatment, with the exception of urine calcium, which was lower in the placebo group (group I).

Biochemistry. There was a significant and marked

increase in serum Ca at 1 and 2 mo in group I patients, with a peak at about 6 wk after the onset of paraplegia (Fig. 1). This change was not found in Cl_2MDP -treated patients at either dose. Serum P elevations were not significantly different, but appeared more marked in placebo patients (Table I). AP levels were unchanged in the three groups.

Urine Ca and OHP were markedly elevated in group I patients, with significant differences when compared with the other two groups at 1, 2, and 3 mo for Ca, and 2 wk and 1 mo for OHP (Figs. 2 and 3). In contrast, there was no significant increase in these parameters in both treated groups, where Ca and OHP remained roughly at about the pretreatment level, even during the follow-up period. The increase in both parameters that occurred 1.5 mo after withdrawal of the drug was not significant, though it was more marked for urine Ca than for OHP. Pretreatment figures were already considerably above normal. Transaminase levels were slightly and briefly elevated in a few cases in the three groups, probably as a result of minimally impaired liver function often seen in paraplegics during the early stages (14). There was no significant difference in leukocyte, platelets, hemoglobin, blood urea nitrogen, creatinine, creatinine clearance, or uric acid between any of the three groups. All these parameters remained within the normal range throughout the study.

Amount of bone (Table II). From pretreatment values of 1.99, 2.00, and 1.98 g/cm for group I, II,

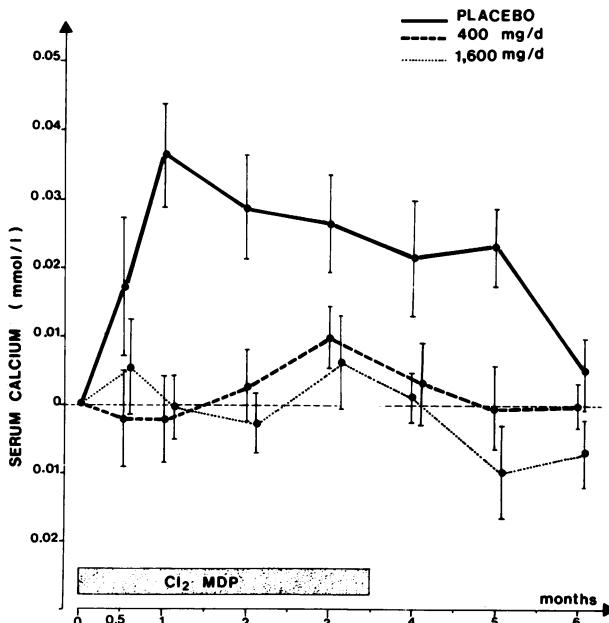


FIGURE 1 Change in serum calcium during treatment and follow-up period (mean \pm SD).

TABLE I
Serum Phosphate and Alkaline Phosphatase Levels during 5 mo Postinjury
(3 mo of treatment, 2 mo of follow-up)

Pretreatment	Months					
	1	2	3	4	5	
Serum phosphate, mg/dl						
placebo	5.1±0.3*	5.4±0.3	5.8±0.2	6.0±0.5	4.7±0.3	4.9±0.3
400	4.9±0.1	5.3±0.4	5.3±0.3	5.3±0.2	5.1±0.3	4.8±0.3
1,600	4.8±0.3	5.6±0.5	5.4±0.1	5.0±0.4	4.6±0.3	5.1±0.3
Serum alkaline phosphatase, Bodansky Units						
placebo	4.9±0.5	4.9±0.5	5.5±0.7	4.0±0.7	4.3±0.8	4.7±0.5
400	4.2±0.1	5.3±0.8	6.2±1.1	5.3±1.4	4.8±1.3	5.25±2.2
1,600	4.5±0.8	5.4±0.9	5.5±1.2	5.5±0.6	4.6±1.1	4.5±1.6

* ±SEM.

and III, respectively, there was a continuous and significant decrease only in the placebo group (group I): 1.85 gm/cm at 6 mo, as opposed to 2.02 and 1.97 g/cm in groups II and III.

The femur radiogrammetry showed a marked mean widening of the endosteum in group I: +0.81 mm, no change in group III: +0.01 mm, and a narrowing of the endosteum in group II: -0.17 mm ($P < 0.05$). There was consequently a significant inhibition of the loss of

cortical width in group II (m: +0.10 mm), as compared with the placebo group (m = -0.48 mm). In group III, there was a slight decrease of cortical width, resulting from an endosteal widening associated with some periosteal narrowing.

Bone histomorphometry (Tables III and IV). In the three groups, TBV and total bone density (TBD) decreased moderately, but not significantly. There was an almost identical reduction from the initial value of TBV: -21% in group I (placebo), -24% in group II (400 mg), -24.2% in group III (1,600 mg) for values obtained with the manual method, and

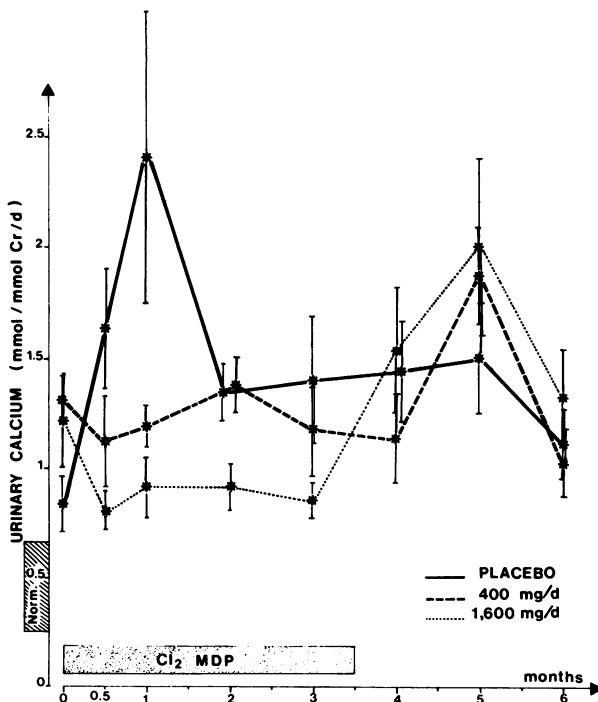


FIGURE 2 Evolution of urinary calcium during treatment and follow-up period (mean±SD).

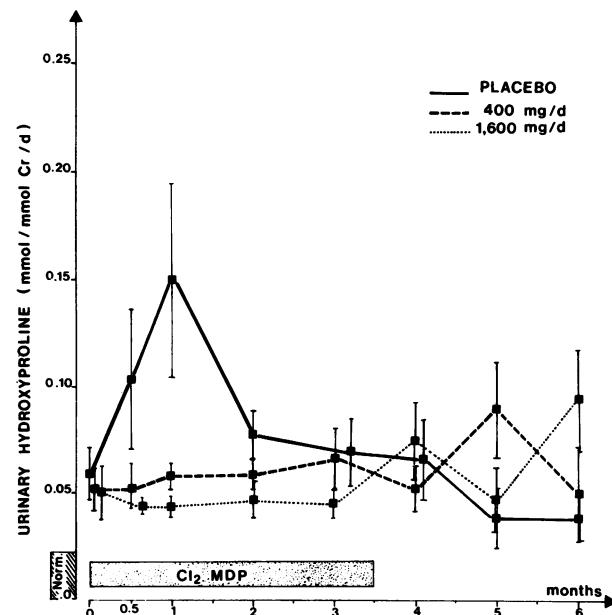


FIGURE 3 Evolution of urinary hydroxyproline during treatment and follow-up period (mean±SD).

TABLE II
*Changes in Bone Density Measurements at the Lower End of Tibia (Absorptiometry)
 and Midshaft of Femur (Radiography)*

	Bone mineral content			Periosteal width (mean difference between 0 and 3.5 mo, in mm)	Endosteal width	Cortical width
	Pretreatment	3 mo	6 mo			
Group I (placebo)	1.99±0.01*	1.87±0.02	1.85±0.04	–0.14	+0.81	–0.48
		†		†		
Group II (Cl ₂ MDP, 400 mg)	2.00±0.04	1.92±0.03	2.02±0.06	+0.04 (P < 0.05)	–0.17 (P < 0.05)	+0.10
Group III (Cl ₂ MDP, 1,600 mg)	1.98±0.05	1.93±0.04	1.97±0.05	–0.47	+0.01	–0.18

* SEM.

† P < 0.05.

–25.2, –26.5, and –18.7% for values obtained with the image analyzer. The reduction of mean TBD was –13.1, –16.1, and –24.4%, respectively, but these differences were not significant.

There was a slight, but insignificant increase in resorption surfaces in the three groups. An increase was also observed in the number of osteoclasts per mm²: +48% in group I, +17% in group II, and +27% in group III. The mean numbers of osteoclasts in each group were already above normal values before treatment.

The size of the periosteocytic lacunae was not significantly different in the treated and untreated groups at the end of treatment, although it was slightly above the upper normal limit (N: 50.7±0.8 μm²), in group III (58.1±2.3 μm²).

A decrease in osteoid volume during the early stages after spinal cord injury has been reported (1), and pretreatment values of osteoid volume and surfaces and osteoid seam thickness index (TIOS) did not appear particularly low in this study. In the posttreatment biopsies, TIOS was significantly increased in group III when compared with group I.

The mean calcification rate, measured only in the three cases demonstrating double labels in trabecular bone, was 0.60 μm/d in group I at the end of treatment. In groups II and III, it was 0.47±0.5 μm/d (n = 5) and 0.43±0.10 μm/d (n = 6), respectively. Interestingly, there were four cases with total absence of labeling in group I, one in group II, and none in group III. It thus appears that there is a more rapid return to a normal osteoid index under treatment, and no reduction in the

TABLE III
Effect of Cl₂MDP on Bone Histomorphometry: Amount of Bone and Resorption Parameters

	Group I (placebo)		Group II (Cl ₂ MDP 400 mg/d)		Group III (Cl ₂ MDP 1,600 mg/d)	
	Before	100th d	Before	100th d	Before	100th d
Trabecular bone volume, %						
manual method	22.8±1.9*	17.9±2.3	24.1±3.3	18.2±1.1	25.2±3.1	19.1±2.2
image analyzer	21.0±1.1	15.7±2.0	23.8±3.8	17.5±0.6	23.0±5.2	18.7±2.0
	21.4±2.0§		22.0±2.3§		23.3±1.8§	
Total bone density, %	44.0±2.1	38.6±3.5	45.4±4.9	38.1±3.3	46.2±6.1	34.9±3.1
(image analyzer)	34.9±2.6§		37.8±1.3		39.5±0.9	
Total resorption surf, %	8.5±1.0	9.2±0.9	6.4±1.0	9.9±1.2	5.5±1.3	9.4±2.9
(n: 3.6±0.1)†						
Osteoclasts per mm ² trabecular bone section (n < 0.20)	0.46±0.14	0.68±0.17	0.29±0.05	0.34±0.04	0.32±0.05	0.44±0.14

* SEM.

† No change with age and sex.

§ Mean normal values for matching age and sex distribution.

TABLE IV
Effect of Cl_2MDP on Bone Histomorphometry: Osteoid Tissue and Periosteocytic Lacunae

	Group I (placebo)		Group II (Cl_2MDP 400 mg/d)		Group III (Cl_2MDP 1,600 mg/d)	
	Before	100th d	Before	100th d	Before	100th d
Trab. osteoid volume, % (n: 10–49 yr = ♂ 3.3±0.3; ♀ 2.1±0.3)	1.4±0.2*	2.4±0.9	0.8±0.2	1.6±0.3	1.3±0.4	2.6±0.6
Trab. osteoid surfaces, % (n: 10–49 yr = ♂ 16.2±1.3; ♀ 19.1±1.5)	12.9±2.6	14±4.2	9.4±2.2	12.4±2.3	9.1±1.5	13.8±2.5
$P < 0.05$						
Thickness index of osteoid seams (n: 10–49 yr = ♂ 19.6±0.7; ♀ 18.3±0.6)	12.6±0.6	11.6±2.2	10.1±1.4	14.3±1.5	13.7±3.0	18.8±1.7
Periosteocytic lacunae size (μm^2) (n: 50.7±0.8)†	53.7±2.3	55.3±3.4	53.3±1.8	53.5±3.2	51.8±2.6	58.1±2.3

* SEM.

† No change with age and sex.

calcification rate in both treated groups. Thus, Cl_2MDP did not create a mineralization defect in treated bone.

Clinical status. No fractures occurred during treatment. Heterotopic ossifications, a common complication after spinal cord injury, were observed in six cases, two in each group. Urinary lithiasis appeared in six cases: three in group I, one in group II, and two in group III. The incidence of lithiasis in the treated groups may follow from the posttreatment rebound in urinary calcium (Fig. 2). No clinical side-effects were noted during treatment, particularly in the gastrointestinal tract.

DISCUSSION

Cl_2MDP prevents the elevation of serum Ca commonly encountered in recent paraplegic patients, and it also counteracts a further release of Ca and OHP in urine. In contrast to the changes noted in the placebo group, the group of patients treated with a daily dose of 400 mg of Cl_2MDP showed no reduction during treatment in the amount of bone as determined by both absorptiometric and radiogrammetric measurements. These results, obtained by noninvasive methods of measurement of appendicular bone, suggest an inhibition of the increased osteoclastic activity usually noted in bones of recent paraplegic patients over the first six postinjury months (1). These data are thus consistent with the inhibiting effect on increased osteoclastosis seen by Meunier (4) and Douglas (16) in Paget's disease of bone, by Siris (17) on multiple

myeloma, and Chapuy (18) on bone metastases with hypercalcemia.

But the changes in the histomorphometric parameters reflecting the amount of bone (trabecular bone volume and total bone density) in the axial skeleton (pelvis) are not in agreement with the remaining data. This is in fact not surprising because there is no established correspondence between axial and peripheral bone (19).

In addition, the increase in the number of osteoclasts per square millimeter, despite a greater increase in the mean of the placebo group, did not show significant differences between the three groups.

Two factors might explain this discrepancy. Firstly, our pretreatment values indicate clearly that from the time when the patients were included in the study, the histomorphometric changes already observed reflected an earlier and greater increase in resorption parameters than we might have expected from the data collected in a previous study (1). This early increase in the resorption parameters may have biased our baseline pretreatment values for parameters reflecting both the amount of bone and the bone remodeling prints.

Secondly, serum iPTH was unfortunately not measured because parathormone levels are known to be normal or low in paraplegic patients (20), and we were not at that time aware of the increase in iPTH noted in pagetic patients treated by Cl_2MDP (4, 16) or APD (21). The increase in the size of the periosteocytic lacunae with the 1,600-mg dose might have been due to an increased parathyroid secretion (22). Tra-

pecular osteoid surfaces were markedly increased under treatment: +51.6% with the 1,600-mg dose and +31.9% with 400 mg as compared with +8.5% in the placebo group. These surfaces were active in all patients treated with 1,600 mg of Cl_2MDP where double labeling was interpretable (6, 7). In contrast, only three patients of the placebo group had some tetracycline labeling at the time of their second posttreatment biopsy. Alkaline phosphatases were slightly increased under treatment, but the presence of two cases of heterotopic ossification in each group makes any comparison between the three groups difficult. These results are therefore only suggestive of increased iPTH and do not constitute a definite proof. Increased parathormone levels might, however, explain some discrepancies noted in our data, especially between the two treated groups.

It is also possible that the effect of Cl_2MDP was decreased by the small amount of osteoid tissue available in the early stages of paraplegia, because diphosphonates are believed to be adsorbed first at the surface of hydroxyapatite before being ingested by osteoclasts.

The significant decrease in urinary calcium excretion under treatment and its rebound after withdrawal of the drug suggest an independent effect of Cl_2MDP on calcium handling by the kidney, though increased parathormone levels might also account for such renal changes.

Heterotopic ossification appears uncontrolled by Cl_2MDP treatment. This is to be expected from the absence of an adverse effect of Cl_2MDP on mineralization both *in vitro* (3) and in this study. Heterotopic ossification is a severe complication of paraplegia. Now ethane-1 hydroxy-1, 1 diphosphonate (EHDP) has been recommended (23) as a preventive treatment for heterotopic ossification, or for its recurrence after surgery, though EHDP appears ineffective in the prevention of the acute bone loss of immobilized patients (24). The different properties of these two diphosphonates therefore suggest a test of the simultaneous use of EHDP and Cl_2MDP in such patients.

As far as the dose-dependent effects were concerned, 400 mg of Cl_2MDP given daily provided better results on absorptiometric and radiogrammetric measurements than 1,600 mg. The 1,600-mg dose, however, was slightly more effective on the biochemical parameters (serum and urine Ca, OHP), but there was no mineralization defect with either dose. It thus seems that a dose of 800 mg/d should be tested. It also appears that, in the case of paraplegia, the effect of Cl_2MDP is closely related to the delay of administration of the drug. Possibly the treatment would have been more effective if given immediately after the onset of paraplegia, intravenously if necessary.

In conclusion, Cl_2MDP treatment appears capable of controlling the biochemical parameters reflecting the acute metabolic bone changes occurring soon after a sudden onset of paraplegia, and to prevent the loss of bone. However, its inhibitory effect on the increased osteoclastic resorption seen in recent paraplegics was not demonstrated in this study over a treatment period of 14 wk, and at doses of 400 and 1,600 mg/d. Nevertheless, its use would appear to show promise at least in reducing the hypercalcemia and urinary excretion of calcium and hydroxyproline that results from immobilization (25), with little effect on bone mineralization.

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