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THE EFFECT OF 1α-HYDROXYCHOLECALCIFEROL ON THE HEALING OF EXPERIMENTAL FRACTURES IN ADULT RATS

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Unilateral tibial fractures were produced in adult, 1-year-old, male Sprague-Dawley rats. The animals were then treated for 6 weeks with daily doses of 2.5 μg, 1.25 μg or 0.125 μg 1α-hydroxycholecalciferol (1α-OH-D_3). The aim of the investigation was to study the effect of this treatment on the healing process of the fracture and on the composition of the fractured bone.

The general effect of 2.5 μg of 1α-OH-D_3 was a significant loss of body weight (20 per cent) and hypercalcaemia. The lower dose levels, however, did not affect the body weight, and with a dose of 0.125 μg the serum calcium level did not increase significantly.

The healing rate of the fractures increased in all treatment groups as compared with the controls. The water content of the fractured tibias increased in the rats treated with 2.5 μg doses but decreased in the other groups. On the other hand the mineral content increased in the groups treated with 1.25 μg and 0.125 μg doses and decreased in the largest dose group. Furthermore the amount of organic material per wet weight increased with the 2.5 μg dose and was mainly unchanged in the other groups. The hydrated bone density and the cortical thickness of the tibia increased most significantly in the group treated with 0.125 μg but the trabecular bone area of the periosteal callus did not increase significantly.

The conclusion is drawn that treatment with small doses of 1α-OH-D_3 has a beneficial effect on the healing rate and on the mineralization of the fracture callus, and on cortical bone formation.

Key words: fracture healing; experimental; 1α-hydroxycholecalciferol

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Therapeutic doses of Vitamin D promote calcification of bone in rickets and osteomalacia but Compere et al. (1939) found that treatment with physiological doses of Vitamin D did not exert any stimulating effect on the osteogenesis or the mineralization of callus in experimental fractures. Beneficial effects of Vitamin D on fracture healing have, however, been reported in other studies (Bors 1927, Collazo et al. 1930). Moreover, Vitamin D has been found to stimulate the differentiation of the osteogenic cell layer of the periosteum of the radius in adult guinea-pigs during fracture healing (Grauer 1932). Vitamin D was also found to display a special selective effect on the organic phase of bone healing in rats (Udupa & Prasad 1963) and on the mineralization of autogenous bone grafts in rabbits (Donatelli et al. 1962) and further to accelerate the initial mineralization in fracture repair of rats (Steier et al. 1967).

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Great advances in the research of the biological functions and metabolism of Vitamin D have taken place in recent years. Thus, Vitamin D_3 is known to be hydroxylated in the liver to 25-hydroxycholecalciferol (Ponchon & DeLuca 1969) and further in the kidney to the metabolically active form 1.25-dihydroxycholecalciferol (Fraser & Kodicek 1970). The synthetic analog 1α-hydroxycholecalciferol (Holick et al. 1973) which is converted to 1.25-dihydroxycholecalciferol in the organism (DeLuca et al. 1976) has been found to increase the intestinal absorption of calcium (Peacock et al. 1974) and thus secondarily affect bone resorption (Raisz et al. 1972) and remodelling.

Because the observations made in previous studies on the effect of Vitamin D are inconclusive, and because the dose-related effects of Vitamin D on fracture healing have not been thoroughly examined, the present investigation was undertaken to study the effect of various doses of the synthetic drug 1α-OH-D_3 on healing of experimental fractures in adult rats.

MATERIAL

Altogether 75 male adult rats of the Sprague-Dawley strain were used in two different series. The rats were about 1-year-old. The initial body weight of the animals in the first series was 473 g, in the second 538 g. All rats received a diet containing about 1.15 per cent calcium, 0.8 per cent phosphorus, and 24.5 per cent protein per dry weight. The diet contains an adequate amount of Vitamin D, i.e. 15 IU Vitamin D_3/10 g diet. The animals received food and water *ad libitum*.

Five days a week the rats received peroral doses of 1α-OH-D_3 diluted in propylenglycol*. The administration of the drug was started after the tibia and fibula were fractured and continued for 6 weeks, after which the animals were sacrificed.

The *first series* comprised 30 rats. Thirteen were treated with 2.5 μg 1α-OH-D_3/day, which corresponds to a dose of approximately 5 μg/kg body weight. The remaining animals were used as untreated controls.

The *second series* comprised 45 rats. Fifteen rats were treated with 1.25 μg, and another fifteen with 0.125 μg 1α-OH-D_3/day, which corresponds to doses of 2.5 μg and 0.25 μg/kg body weight, respectively. The remaining animals in this series were used as controls.

* Kindly supplied by Löven Ltd, Copenhagen, Denmark

METHODS

The *final body weights* of all rats were recorded. Seventy-two hours before killing 50 mg of Terramycin® was injected into each animal intraperitoneally. The rats were sacrificed by bleeding from the femoral artery under ether anaesthesia. Blood was drawn for determination of *total serum calcium*. The consolidation of the fracture was tested manually. The right fractured tibia was cleaned from the surrounding soft tissues and was subjected to various analyses. Roentgenograms were taken, and the "*tibia score*" representing the cortical bone thickness was measured on the mid-shaft of the tibia. The whole fractured tibia including the fracture area and the periosteal callus lump was used for determination of *wet and dry weights, hydrated bone density* and *total ash content* according to methods previously described and used in the laboratory (Sevastikoglou 1972).

Five fractured tibias from each group were prepared for histological, microradiographic and fluorescence labelling analyses. The *histologic* samples were stained with haematoxylin-eosin and cut into 7 μ thick sections after decalcification. The *microradiographs* were prepared after embedding in methylmethacrylate and sawing off about 80 μ thick slices. These samples were also examined for fluorescence labelling under UV-light in a Zeiss microscope. For quantitation of trabecular bone mass of the periosteal callus a Zeiss image auto-analyzer was used.

The significance of observed differences was computed using the *t*-test for non-paired experiments.

RESULTS

No rats died during the experiment. The oral administration of the drug succeeded without difficulty.

The *final body weight* of rats treated with 2.5 μg 1α-OH-D_3 showed a significant decrease ($P<0.001$) as compared with their initial body weight and with the final weight of the other rats in this series, which did not change significantly (Table 1).

The *consolidation of the fracture*, tested

Table 1. Final body weight and serum calcium level in rats treated over a period of 6 weeks with 2.5 μg, 1.25 μg and 0.125 μg 1α-OH-D_3/day, respectively

	Series I		Series II		
	Control rats	Experimental rats (2.5 μg 1α-OH-D_3)	Control rats	Experimental rats (1.25 μg 1α-OH-D_3)	(0.125 μg 1α-OH-D_3)
Final body weight (g)					
n	17	13	15	15	15
$\bar{y}$	492	403	535	540	555
$s\bar{y}$	23	61	63	15	36
P	—	<0.001	—	> 0.05	> 0.05
Serum calcium mmol/l					
n	8	8	10	10	10
$\bar{y}$	2.52	3.25	2.63	2.92	2.68
$s\bar{y}$	0.06	0.01	0.08	0.12	0.67
P	—	<0.001	—	<0.001	>0.05

manually at the end of the experiment, gave the following results: in rats treated with 2.5 μg 1α-OH-D_3 the fracture was stable in 6/13 cases and in the control rats in 3/17 cases. In rats treated with 1.25 μg, 7/15, and those treated with 0.125 μg, 6/15 fractures were stable. In the control rats of both treated series stable fractures were found in 4/15 cases.

The *total serum calcium* was significantly increased ($P<0.001$) in rats treated with 2.5 μg and 1.25 μg 1α-OH-D_3, while no significant increase in serum calcium was observed in rats treated with 0.125 μg 1α-OH-D_3 as compared with the corresponding values from the control groups of animals (Table 1).

The *wet weight* of the fractured tibia including the callus lump increased in all treated rats, but the increase was significant ($P<0.01$) only in rats treated with 2.5 μg of 1α-OH-D_3 (Tables 2 and 3).

The *dry weight* of the fractured tibia increased in all treated rats as compared with the controls. A significant increase ($P<0.05$) was found in rats treated with 0.125 μg of 1α-OH-D_3 (Tables 2 and 3).

The *hydrated bone density* of the fractured tibia increased significantly ($P<0.05$) in rats treated with 1.25 μg and 0.125 μg 1α-OH-D_3, respectively (Tables 2 and 3).

The *total ash content* of the fractured tibia increased in all treated rats. The increase was significant ($P<0.01$) in the rats treated with 0.125 μg of 1α-OH-D_3 (Tables 2 and 3).

The "*tibia score*" increased in all treated rats as compared with the controls. The increase was significant ($P<0.05$) in rats treated with 2.5 μg and 0.125 μg 1α-OH-D_3, respectively (Tables 2 and 3).

The *trabecular bone area* of the periosteal callus showed an increase, though not significant, in rats treated with 0.125 μg 1α-OH-D_3 as compared with the controls. The values from rats in the other groups decreased. The differences were non-significant (Tables 2, 3 and Figure 1).

The *histological* appearance of the callus from rats of different treatment groups and the controls showed no qualitative differences.

The *fluorescence labelling* of the osseous callus did not differ between the animals from the various treatment groups and the controls.

Table 2. Parameters of fractured tibia in rats treated over a period of 6 weeks with 2.5 μg of 1α-OH-D_3/day

		Series I	
Fractured tibia		Control rats	Experimental rats (2.5 μ 1α-OH-D_3)
Wet weight (g)	n	12	8
	$\bar{y}$	1.0620	1.3050
	$s\bar{y}$	0.1140	0.1940
	P	—	<0.01
Dry weight (g)	n	12	8
	$\bar{y}$	0.7910	0.9130
	$s\bar{y}$	0.0560	0.1350
	P	—	>0.05
Hydrated bone density (w/v)	n	12	8
	$\bar{y}$	1.5146	1.4723
	$s\bar{y}$	0.0460	0.0430
	P	—	>0.05
Total ash content (g)	n	12	8
	$\bar{y}$	0.5282	0.5796
	$s\bar{y}$	0.0428	0.0807
	P	—	>0.05
"Tibia score" (%)	n	12	8
	$\bar{y}$	36.9	40.9
	$s\bar{y}$	3.2	5.4
	P	—	<0.05
Trabecular bone area of periosteal callus (%)	n	5	5
	$\bar{y}$	23.67	16.64
	$s\bar{y}$	12.99	8.04
	P	—	>0.05

DISCUSSION

The present experiments indicated that large doses of 1α-OH-D_3, i.e. 2.5 μg, produced a significant hypercalcaemia and additionally decreased the body weight of the rats by about 20 per cent. This dose and still larger doses probably produce toxic effects in the adult rat. Dose levels of 1.25 μg did not significantly affect the body weight but the serum calcium level still increased significantly producing slight hypercalcaemia as compared with the controls. Lower dose levels, i.e. 0.125 μg, did not influence the body weight of the animals and their serum calcium levels rose only slightly, and not significantly, during treatment. This low dose level probably does not produce any toxic effects in the adult rat.

In addition to these general effects there were also specific alterations in the composition and in the other examined parameters of the fractured tibia and the periosteal callus during treatment with 1α-OH-D_3. An acceleration of the consolidation rate of the fracture tested manually was recorded in all treatment groups compared with the controls. Furthermore the mineralization of the fractured bone increased as evidenced by the total ash content and by the amount of mineral, expressed as a percentage of the wet weight, when treated with the low dose

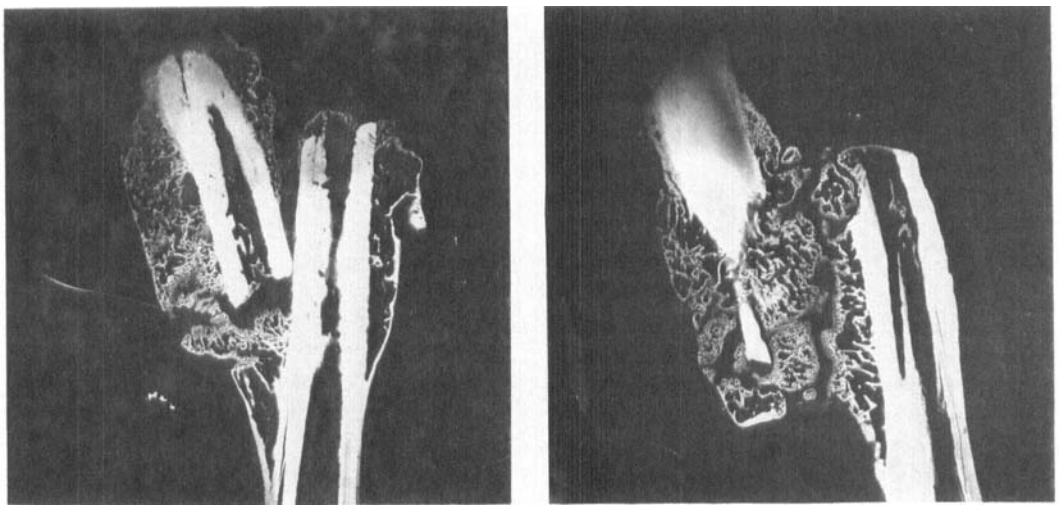
Table 3. Parameters of fractured tibia in rats treated over a period of 6 weeks with 1.25 µg and 0.125 µg of 1α-OH-D_3/day, respectively

Series II				
Fractured tibia		Control rats	Experimental rats (1.25 µg 1α-OH-D_3)	(0.125 µg 1α-OH-D_3)
Wet weight (g)	n	10	10	10
	$\bar{y}$	1.2839	1.3063	1.3812
	$s\bar{y}$	0.1521	0.1188	0.1221
	P	—	>0.05	>0.05
Dry weight (g)	n	10	10	10
	$\bar{y}$	0.8015	0.8468	0.8831
	$s\bar{y}$	0.0783	0.0623	0.0712
	P	—	>0.05	<0.05
Hydrated bone density (w/v)	n	10	10	10
	$\bar{y}$	1.4120	1.4450	1.4390
	$s\bar{y}$	0.0250	0.0320	0.0240
	P	—	<0.05	<0.05
Total ash content (g)	n	10	10	10
	$\bar{y}$	0.4974	0.5330	0.5570
	$s\bar{y}$	0.0444	0.0363	0.0463
	P	—	>0.05	<0.01
"Tibia score" (%)	n	15	15	15
	$\bar{y}$	44.18	45.92	46.61
	$s\bar{y}$	2.88	1.75	2.86
	P	—	>0.05	<0.05
Trabecular bone area of periosteal callus (%)	n	5	5	5
	$\bar{y}$	31.50	29.87	32.40
	$s\bar{y}$	11.26	14.17	14.74
	P	—	>0.05	>0.05

levels. These observations are partly supported by similar results achieved by Vitamin D_2 treatment in which the uptake of ^{32}P and thus the mineralization rate of the healing of autogenous bone grafts in rabbits was enhanced (Donatelli et al. 1962). Furthermore the ash content of the rat callus was observed to increase as a result of Vitamin D_2 treatment (Steier et al. 1967).

The effect of 1α-OH-D_3 was also an increase in wet and dry weights of the fractured bone indicating an increase of organic matrix components. When calculating the amount of water in per cent of wet weight it was noted that there was an increase in water content of the fractured bone treated with 2.5 µg doses. In contrast the water percentage diminished in the fractured tibias treated with the other dose levels. The organic material expressed as a percentage of the wet weight increased slightly in the group treated with 2.5 µg and was mainly unchanged in the other groups. On the other hand the total dry weight increased significantly in the group treated with 0.125 µg of 1α-OH-D_3. In a previous study it has been observed histologically that Vitamin D_2 accelerates the organic phase of fracture repair (Udupa & Prasad 1963).

At all dose levels of 1α-OH-D_3 the capacity to increase the cortical bone thickness in the tibia adjacent to the fracture was demonstra-



A B

Figure 1. Microradiograph of tibial fractures after 6 weeks treatment with 1α-OH-D_3. A control, B treatment with 0.125 μg dose. The differences are small, but the bone trabeculae are stronger in the treated group compared with the control.

ted. This can probably be seen as an increase in either periosteal or endosteal new bone formation. The hydrated bone density, however, decreased when treated with large doses and increased with the lower dose levels. The trabecular bone area of the periosteal callus did not increase significantly in the different treatment groups, although at the low dose level, i.e. 0.125 μg, there was a bone increasing effect.

The conclusion drawn from these experiments is that the dose level of 0.125 μg of 1α-OH-D_3 can best promote healing of rat fractures. It increases the mineral in fractured bone and enhances the cortical bone mass as well as the periosteal callus without producing hypercalcaemia or significantly affecting the body weight of the animals.

This beneficial effect of low doses of 1α-OH-D_3 may be explained by a direct action on the bone producing cells. There is some evidence that metabolites of Vitamin D_3 may act via physiological protein receptors in bone cells (Kream et al. 1977). On the other hand there may be a secondary effect on bone due to the ability of 1α-OH-D_3 to increase the intestinal absorption rate of calcium (Peacock et al. 1974). The increase in the serum calcium level may inactivate the parathyroid glands and thus decrease the activity of the osteoclasts allowing bone forming cells to activate and increase both the mature bone mass and the formation of callus bone.

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