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EFFECTS OF A NOVEL VITAMIN D ANALOGUE MC 903 ON CELL PROLIFERATION AND DIFFERENTIATION *IN VITRO* AND ON CALCIUM METABOLISM *IN VIVO*

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Abstract—MC 903 is a novel vitamin D analogue which has been tested for its effects on cell differentiation and cell proliferation *in vitro* using the human histiocytic lymphoma cell line U937, and on calcium metabolism in rats *in vivo*. In the present investigation MC 903 was compared to the natural metabolite of vitamin D_3, 1α,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$] and to its synthetic analogue 1α-hydroxycholecalciferol [$1\alpha(OH)D_3$]. MC 903 was found to be a potent inducer of cell differentiation and to inhibit cell proliferation and DNA-synthesis in concentrations comparable to those observed with $1,25(OH)_2D_3$. $1\alpha(OH)D_3$, which is only active after metabolic conversion to $1,25(OH)_2D_3$, was more than 100 times less potent.

Oral or intraperitoneal administration of MC 903 to rats showed that the compound was at least 100 times less active than $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$ in causing hypercalciuria, hypercalcemia and bone calcium mobilisation. The low vitamin D activity of MC 903 was further confirmed by administration of the compound to rachitic rats. The strong direct effects of MC 903 on cell proliferation and cell differentiation, coupled with its decreased activity as a classical vitamin D makes this compound an interesting candidate for studies in human proliferative disorders such as psoriasis.

In recent years the role of 1α,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$] as an important factor in the regulation of bone and mineral metabolism has been firmly established [1, 2]. $1,25(OH)_2D_3$ is the active form of vitamin D_3 and is formed by consecutive hydroxylations of the latter compound in the liver and in the kidney (see Fig. 1). $1,25(OH)_2D_3$ acts by regulating calcium and phosphate metabolism primarily by effects on intestine, bone and kidney. 1α-Hydroxycholecalciferol [$1\alpha(OH)D_3$], a synthetic analogue of vitamin D_3 which bypasses the kidney 1α-hydroxylase but is dependent on 25-hydroxylation in the liver to form $1,25(OH)_2D_3$, shows the same profile of activity [3]. These two derivatives of vitamin D_3 are widely used in the treatment of various disorders of vitamin D metabolism such as renal osteodystrophy, hypoparathyroidism and vitamin-D-resistant rickets [2].

$1,25(OH)_2D_3$ mediates its effects on intestinal uptake of calcium and phosphate by binding to a steroid type receptor present in cells from the intestinal epithelium [4]. Specific receptors for $1,25(OH)_2D_3$ have recently been found in tissues such as skin, muscle, pancreas [5, 6, 7] and in some tumours [8] and receptor-mediated effects on cell proliferation and cell differentiation have been observed [9–11]. The recent observation that cultured cells from psoriatic skin exhibit a partial resistance to the effects of $1,25(OH)_2D_3$ on cell proliferation and differentiation and that this resistance can be overcome by large increases in $1,25(OH)_2D_3$ concentrations [12] suggest that vitamin D derivatives may offer a new approach to the therapy of psoriasis—a disease characterized by abnormal increases in epidermal cell turnover [13]. In support of this theory a number of recent reports have demonstrated beneficial effects of topical or systemic treatment of psoriasis with $1,25(OH)_2D_3$ or $1\alpha(OH)D_3$ [14, 15].

Unfortunately, the use of highly active vitamin D_3 derivatives to control cell proliferation and promote cell differentiation is limited by their potent effects on calcium metabolism. Hypercalcemia is induced by systemic doses higher than a few μg per day, and topical application is complicated by the risk of transdermal absorption of the active compound in areas of psoriatic skin lesions.

It has therefore been our aim to develop new vitamin D_3 analogues with potent effects on cell proliferation and cell differentiation, but with a lower risk of inducing the classical vitamin D-associated side effects: hypercalciuria, hypercalcemia and induction of bone resorption. As a result of our chemical and pharmacological research a new vitamin D_3 analogue MC 903 has been selected for clinical studies in patients with psoriasis. Figure 1 shows the chemical structure of MC 903 and its relationship to $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$. The chemistry and synthesis of MC 903 will be described in detail elsewhere [16].

This paper describes the *in vitro* effects of MC 903 on induction of cell differentiation and inhibition of cell proliferation, using the human histiocytic lymphoma cell line U937 [17]. This cell line displays high-affinity receptors for $1,25(OH)_2D_3$ [18]. The effects of MC 903 on calcium metabolism *in vivo* after p.o. and i.p. administration to rats were also investigated. In all the studies MC 903 was compared with $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$.

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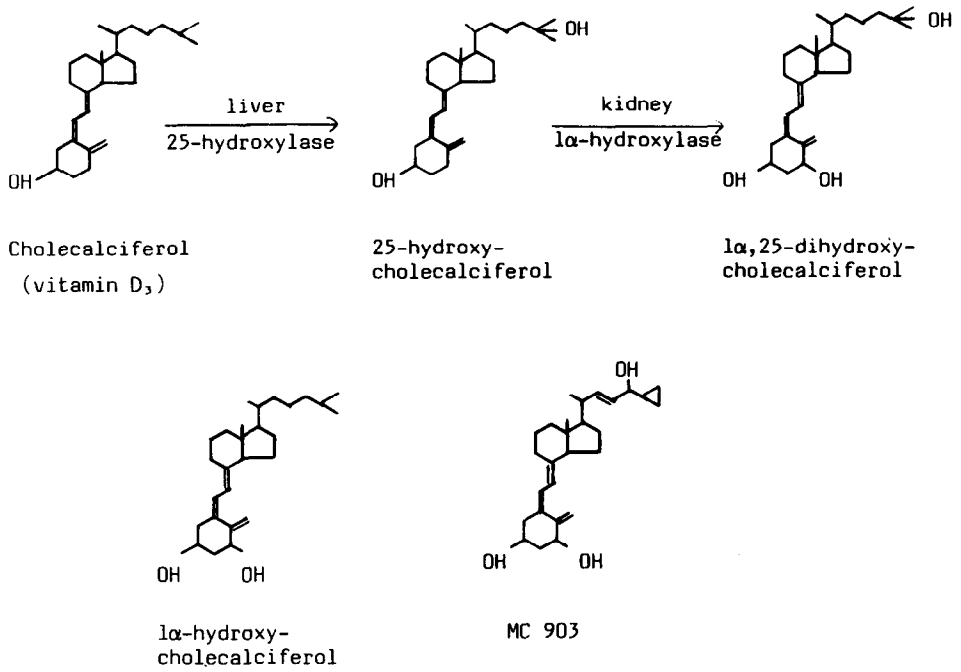

Fig. 1. Formation of $1,25(OH)_2D_3$ by hydroxylation of cholecalciferol in the liver and kidney. Chemical structures of the related vitamin D_3 analogues $1\alpha(OH)D_3$ and MC 903.

MATERIALS AND METHODS

Compounds. 1α-25-Dihydroxycholecalciferol [$1,25(OH)_2D_3$] was obtained from Duphar, Holland. 1α-Hydroxycholecalciferol [$1\alpha(OH)D_3$] and MC 903 were synthetized in the Department of Chemical Research, Leo Pharmaceutical Products.

The compounds were dissolved in propylene glycol for the *in vivo* studies and in abs. ethanol for the *in vitro* studies.

Cellular effects in vitro. The human histiocytic lymphoma cell line U937 was propagated *in vitro*, with twice-weekly passages, in medium RPMI 1640 (25 mM HEPES, Gibco), containing 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin and 10% fetal calf serum (Gibco).

The binding of the vitamin D derivatives to the cytosolic receptor for $1,25(OH)_2D_3$ was performed according to the methodology described by Manolagas and Deftos [19]. U937 cells were washed with serum-free RPMI 1640 and adjusted to 1×10^6 cells per 0.15 ml of the same medium. The cells were incubated with 1.0 nM [^{3}H]-$1,25(OH)_2D_3$ (180 Ci/mmol, TRK.656, Amersham, U.K.) for 60 min at 37°. Aliquots of 0.15 ml cells were then transferred to tubes containing increasing concentrations of $1,25(OH)_2D_3$ or MC 903 (10^{-10} to 10^{-7} M) or $1\alpha(OH)D_3$ (10^{-7} to 10^{-4} M). Prior to addition of the cells, the solvent of the various compounds was evaporated under N_2. After incubation for 60 min at 37° the cells were washed, lysed with hypertonic buffer and centrifuged for 30 min at 80,000 *g*. Supernatants were counted for radioactivity and the concentration giving half maximal displacement of [^{3}H]-$1,25(OH)_2D_3$ was calculated for each compound.

For studies on cell proliferation and cell differentiation U937 cells were adjusted to 1×10^5 cells/ml in medium RPMI 1640, containing 10% fetal calf serum. The cells were plated at 5 ml in small tissue-culture flasks (Nunc Cat. No. 163371, Denmark) and incubated for 96 hr at 37° in 5% CO_2 in air. $1,25(OH)_2D_3$ or MC 903 (0.5×10^{-9} to 10^{-7} M) or $1\alpha(OH)D_3$ (10^{-7} to 10^{-4} M) were present during the whole period of incubation. Control cultures receiving 0.2% ethanol were run in parallel. At the end of incubation non-adherent cells were collected, counted and assayed for cell survival by the Eosin Y exclusion method. A sample of cells from each culture was fixed with formalin and assayed for the presence of membrane-associated non-specific esterase [20]. Cells adherent to the bottom of the culture flasks were likewise fixed and stained.

Cell cultures for assay of [^{3}H]-thymidine ([^{3}H]-TdR) incorporation were plated in multidishes (Nunc Cat. No. 169590, Denmark), 1×10^5 cells/ml, 0.5 ml/well. The dishes were incubated for 96 hr at 37° in the presence of the various vitamin D derivatives and [^{3}H]-TdR was added for the last 4 hr of culture (1 μCi/ml, 5 Ci/mmol, TRA.120, Amersham, U.K.). After labelling the cells were aspirated from the culture wells into ice-cold 12% trichloroacetic acid, and the amount of radioactivity incorporated in the DNA was determined as previously described [21]. Results were expressed as % inhibition of [^{3}H]-TdR incorporation in treated cultures compared to control cultures. Six separate cultures were made for each concentration tested.

Effects on calcium metabolism in vivo. Female inbred Lewis rats (140–150 g) were treated with $1,25(OH)_2D_3$ (0.5 μg/kg/day), $1\alpha(OH)D_3$ (1.0 μg/

kg/day) or MC 903 (1.0, 10 and 100 μg/kg/day) for 7 days. The compounds were administered p.o. or i.p., control rats were given propylene glycol. The rats were placed in metabolic cages, 2 rats per cage, for the period of treatment. Each treatment group consisted of 4–6 rats. The rats received a standard laboratory diet, containing 1% calcium and 0.75% phosphorus, and the food intake was measured daily. Urine was collected daily and blood was collected by cardiac puncture at the end of the experiment for determination of calcium, phosphate and creatinine levels. The tibiae were removed, cleaned of adjacent tissue, dried overnight at 50° and weighed. Metaphyseal bone was obtained as previously described [22] and used for analysis of calcium content.

Total serum calcium was determined by complex formation with *o*-cresolphthalein [23]. Bone fragments were ashed at 800° for 4 hr and dissolved in 1 ml 1 N HCl. Calcium content of bone and urine was determined by titration with Tritriplex VI (EGTA), using a calcium selective electrode (Radiometer, Denmark). Phosphorus and creatinine in serum and urine were determined by standard methods of clinical chemistry.

The results were expressed as mean values ± SD. Statistical analysis was carried out using Student's *t*-test or one-way analysis of variance.

Effects on rachitic rats. Forty to sixty gram rats from a rachitic prone strain (Ph. Nord. IV, 1964) were given a vitamin D-free diet for two weeks whereafter radiograms of the proximal tibia/distal femur were taken. Only rats gaining weight and showing rickets on radiograms were selected for experimentation. Rats were dosed daily with $1,25(OH)_2D_3$ (0.05 and 0.5 μg/kg p.o.) or MC 903 (100 μg/kg p.o.), each group consisting of 5 rats. After 14 days of treatment the rats were X-rayed again and healing of the rickets was compared on the radiograms, using untreated rachitic rats as controls.

RESULTS

Cellular effects of vitamin D derivatives

The ability of the new vitamin D_3 analogue MC 903 to bind to the cellular receptor for $1,25(OH)_2D_3$ was first studied. The human histiocytic lymphoma cell line U937 was chosen for the cellular studies, due to the presence of well-characterized high-affinity receptors specific for $1,25(OH)_2D_3$ in this cell line. The binding assay was performed by displacement of $[^3H]$-$1,25(OH)_2D_3$ from the receptor by adding increasing concentrations of MC 903. To evaluate the binding ability of MC 903 displacement assays were run in parallel with unlabelled $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$. Figure 2 shows that half maximal displacement (displacement of 50% of the bound $[^3H]$-$1,25(OH)_2D_3$) was obtained with MC 903 at 0.5×10^{-9} M, with $1,25(OH)_2D_3$ at 1.2×10^{-9} M and with $1\alpha(OH)D_3$ at a much higher concentration (1.7×10^{-5} M). Thus the affinity of MC 903 for the $1,25(OH)_2D_3$ receptor is at least as high as that of the natural metabolite $1,25(OH)_2D_3$ itself.

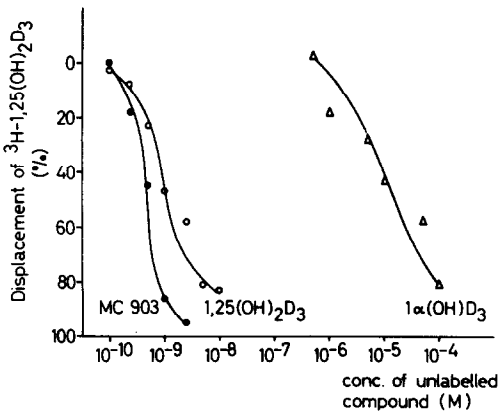

Fig. 2. Receptorbinding of $1,25(OH)_2D_3$, $1\alpha(OH)D_3$ and MC 903. Receptorpositive U937 cells were incubated with 1 nM $[^3H]$-$1,25(OH)_2D_3$ for 60 min at 37°. Increasing concentrations of unlabelled $1,25(OH)_2D_3$, $1\alpha(OH)D_3$ or MC 903 were added, and displacement of $[^3H]$-$1,25(OH)_2D_3$ was measured as % of the amount of radioactivity bound in the absence of added compounds.

In order to establish whether MC 903 is capable of inducing the biological effects of $1,25(OH)_2D_3$ in the U937 cells, i.e. inhibition of cell proliferation and

Table 1. Antiproliferative effects on human histiocytic lymphoma U937 cells

Compound tested	Inhibition of cell proliferation IC_{50} (M)	Viable cells (%)	Inhibition of $[^3H]$-TdR incorporation (%)
$1,25(OH)_2D_3$	2.8×10^{-8} (range $0.4–5.0 \times 10^{-8}$)	>90	57 ± 9
MC 903	1.4×10^{-8} (range $0.1–2.8 \times 10^{-8}$)	>90	49 ± 11
$1\alpha(OH)D_3$	$\sim 10^{-6}$	>90	53 ± 6

U937 cells (1×10^5 cells/ml) were cultured at 37° for 96 hr, in the presence of $1,25(OH)_2D_3$ or MC 903 (at 0.5–100 nM) or $1\alpha(OH)D_3$ (0.1–100 μM). At the end of incubation cell proliferation was determined by cell counting and the inhibitory effects of the 3 compounds were expressed as IC_{50}-values (mean and range of 8 experiments). Cell viability was determined in the same cultures by Eosin Y exclusion. $[^3H]$-TdR incorporation was assessed in separate cultures, labelled with 1 μCi/ml $[^3H]$-TdR during the last 4 hr of culture. Each value is the mean (±SD) of 6 cultures, containing $1,25(OH)_2D_3$ or MC 903 (at 100 nM) or $1\alpha(OH)D_3$ (at 10 μM).

Table 2. Cell differentiation of U937 cells *in vitro*

Compound tested	Conc. in assay (M)	Esterase activity of non-adherent cells % positive cells	Adherence of U937 cells 10^5 cells per culture
None	—	4 ± 3	0.01 ± 0.01
$1,25(OH)_2D_3$	10^{-9}	26 ± 13	0.13 ± 0.01
	10^{-8}	64 ± 24	0.67 ± 0.28
	10^{-7}	80 ± 22	0.91 ± 0.29
MC 903	10^{-9}	38 ± 14	0.12 ± 0.14
	10^{-8}	65 ± 23	0.63 ± 0.36
	10^{-7}	80 ± 15	1.04 ± 0.37
$1\alpha(OH)D_3$	10^{-7}	28 ± 18	0.02 ± 0.01
	10^{-6}	58 ± 24	0.37 ± 0.14
	10^{-5}	79 ± 13	0.43 ± 0.30

U937 cells (1×10^5 cells/ml) were cultured for 96 hr in the presence of $1,25(OH)_2D_3$, MC 903 or $1\alpha(OH)D_3$. At the end of culture non-adherent and adherent cells were stained for esterase activity as a marker of cell differentiation. The % esterase positive cells in the non-adherent cell population was determined and adherent cells were counted. Values represent the mean ± SD of 4–8 separate experiments.

induction of cell differentiation along the monocyte–macrophage pathway, cultures were incubated with MC 903, $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$ for 4 days. Table 1 shows the effects on cell proliferation and DNA-synthesis. Fifty percent inhibition of cell proliferation occurred after incubation with MC 903 at 1.4×10^{-8} M and with $1,25(OH)_2D_3$ at 2.8×10^{-8} M. $1\alpha(OH)D_3$ inhibited cell proliferation at 10^{-6} M. Cell viability was not affected at the end of the culture period, as established by Eosin Y exclusion. DNA-synthesis measured by the incorporation of [^{3}H]-TdR was inhibited approximately 50% by $1,25(OH)_2D_3$ and MC 903 at 10^{-7} M and by $1\alpha(OH)D_3$ at 10^{-5} M.

Table 2 shows the effects of the 3 compounds on the differentiation of U937 cells in culture. Differentiation to cells with monocyte/macrophage characteristics was assessed by observation of cell morphology, cytochemical analysis of the presence of α-naphtyl acetate esterase activity and the appearance of adherent cells in the culture flasks. Control cultures of U937 cells did not exhibit esterase-positive cells, nor did the cells become adherent at the end of the culture period. At 10^{-9} M MC 903 and $1,25(OH)_2D_3$ induced cell differentiation, $1\alpha(OH)D_3$ was effective only at 10^{-7} M. A dose-dependent effect was observed with all 3 compounds.

These results show that the new vitamin D analogue MC 903 is able to bind to the $1,25(OH)_2D_3$ receptor on U937 cells and to exert the biological effects characteristic of $1,25(OH)_2D_3$ itself, in a comparable range of concentrations.

Effects on calcium metabolism

MC 903 was next studied for its effects on calcium metabolism *in vivo* in rats. MC 903 was administered at 1, 10 or 100 μg/kg/day for 7 days, using both p.o. and i.p. administration. Calcium and phosphate excretion in the urine was followed daily and serum calcium and bone calcium content of tibiae metaphyses were measured at the end of the dosing period. The effects of MC 903 were compared to those observed after treatment with $1,25(OH)_2D_3$ (0.5 μg/kg/day 7×) and $1\alpha(OH)D_3$ (1.0 μg/kg/day 7×). These doses are maximum tolerated doses of the 2 compounds, inducing hypercalciuria and hypercalcemia after a few days of dosing.

Table 3. Calcium levels in urine and serum after p.o. administration of vitamin D derivatives

Treatment of rats	Dose μg/kg/day p.o. 7×	Calcium in urine μmol/day Mean ± SD	P-value	Calcium in serum mmol/l Mean ± SD	P-value
Controls	—	17.8 ± 5.0		2.38 ± 0.03	
$1,25(OH)_2D_3$	0.5	135.6 ± 49.7	<0.001	2.73 ± 0.14	<0.001
$1\alpha(OH)D_3$	1.0	143.3 ± 44.6	<0.001	2.74 ± 0.10	<0.001
MC 903	1.0	16.5 ± 5.2	NS	2.40 ± 0.02	NS
MC 903	10.0	18.4 ± 5.8	NS	2.45 ± 0.08	NS
MC 903	100.0	90.3 ± 19.0	<0.001	2.49 ± 0.10	<0.05

Female Lewis rats were treated with $1,25(OH)_2D_3$ (0.5 μg/kg/day), $1\alpha(OH)D_3$ (1.0 μg/kg/day) or MC 903 (1, 10 or 100 μg/kg/day) p.o. for 7 days. Urine was collected daily and serum was obtained at the end of the experiment for the determination of calcium. Calcium concentrations were expressed as μmol/day in urine (N = 10–14) or as mmol/l in serum (N = 4–6). All treated groups were compared to the group of untreated control rats. NS = not significant.

Table 4. Calcium levels in urine and serum after i.p. administration of vitamin D derivatives

Treatment of rats	Dose μg/kg/day i.p. 7×	Calcium in urine μmol/day Mean ± SD	P-value	Calcium in serum mmol/l Mean ± SD	P-value
Controls	—	22.6 ± 11.3		2.35 ± 0.10	
$1,25(OH)_2D_3$	0.5	257.2 ± 112.6	<0.001	2.83 ± 0.16	<0.005
$1\alpha(OH)D_3$	1.0	247.1 ± 126.0	<0.001	3.37 ± 0.27	<0.001
MC 903	1.0	16.4 ± 7.4	NS	2.04 ± 0.27	NS
MC 903	10.0	25.4 ± 9.5	NS	2.25 ± 0.21	NS
MC 903	100.0	206.7 ± 36.0	<0.001	2.61 ± 0.02	<0.005

Female Lewis rats were treated with $1,25(OH)_2D_3$ (0.5 μg/kg/day), $1\alpha(OH)D_3$ (1.0 μg/kg/day) or MC 903 (1, 10 or 100 μg/kg/day) i.p. for 7 days. Determination of calcium in urine and serum was performed as described in the legend to Table 3.

Table 3 shows the mean urinary excretion of calcium and the serum calcium levels after p.o. administration of the 3 compounds. Treatment with $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$ induced a significant increase in urinary calcium (more than 6 times the control level), whereas MC 903 at 1.0 and 10 μg/kg/day had no effect. At 100 μg/kg/day MC 903 increased urinary calcium excretion; however, without reaching the levels observed after treatment with $1,25(OH)_2D_3$ or $1\alpha(OH)D_3$. The same profile of response was seen when serum calcium levels were measured. $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$ significantly induced hypercalcemia (+15% increase in serum calcium), whereas MC 903 only at 100 μg/kg/day increased serum calcium (+5%). Urinary phosphate excretion was also greatly enhanced by $1,25(OH)_2D_3$ at 0.5 μg/kg/day and by $1\alpha(OH)D_3$ at 1.0 μg/kg/day, whereas MC 903 only at 100 μg/kg/day showed an increased excretion of phosphate (results not shown). No changes in bodyweight gain, food intake or creatinine clearance were observed after treatment with any of the 3 compounds in the indicated doses (results not shown).

Table 4 shows the results from the same type of experiment, using i.p. administration of $1,25(OH)_2D_3$, $1\alpha(OH)D_3$ and MC 903 instead of p.o. administration. The effects on calcium in urine and serum were more pronounced after i.p. administration of all 3 compounds, but MC 903 was still only active when administered at 100 μg/kg/day.

Effects on calcium content in tibiae metaphyseal bone are shown in Table 5. Treatment with $1,25(OH)_2D_3$ (0.5 μg/kg/day) or $1\alpha(OH)D_3$ (1.0 μg/kg/day) i.p. for 7 days resulted in a significant decrease in the dry weight and calcium content of the metaphyses. Treatment with MC 903 at 1 or 10 μg/kg/day had no effect, whereas MC 903 at 100 μg/kg/day induced metaphyseal bone weight loss. At this dose a decrease in calcium content of the metaphyses was also observed; this value did not, however, reach statistical significance.

The results show that the systemic effects of MC 903 on calcium metabolism as measured by increased excretion of urinary calcium, increased serum calcium levels and calcium mobilisation from bone were exerted only at doses 100–200 times larger than those required of $1,25(OH)_2D_3$ or $1\alpha(OH)D_3$. In order to supplement these studies on calcium metabolism MC 903 was tested for vitamin D activity in rachitic rats. To ascertain the induction of rickets, all rats were X-rayed prior to treatment. In addition, a group of control and rachitic rats were sacrificed at the end of the experiment and bone calcium content in the tibial proximal metaphyses was measured. In rachitic rats calcium content was decreased by 20% (7.46 ± 0.46 mg calcium/metaphysis, compared to 9.32 ± 0.89 mg/metaphysis in control rats, $P < 0.005$, 5 rats per group). Figure 3 shows the radiograms obtained after 14 days of treatment with MC 903 (100 μg/kg/day p.o.) and $1,25(OH)_2D_3$ (0.05 and 0.5 μg/kg/day p.o.). Treatment with MC 903 at 100 μg/kg or $1,25(OH)_2D_3$ at 0.05 μg/kg was unable to heal the rickets. In contrast $1,25(OH)_2D_3$ at 0.5 μg/kg had a clear healing effect

Table 5. Effects of vitamin D derivatives on bone weight and bone calcium content

Treatment of rats	Dose μg/kg/day i.p. 7×	Tibia metaphyses dry weight (mg) Mean ± SD	P-value	Calcium in bone mg per metaphysis Mean ± SD	P-value
Controls	—	39.4 ± 1.7		9.1 ± 1.0	
$1,25(OH)_2D_3$	0.5	34.9 ± 1.4	<0.01	7.6 ± 0.3	<0.05
$1\alpha(OH)D_3$	1.0	31.3 ± 3.4	<0.01	6.4 ± 0.3	<0.005
MC 903	1.0	38.2 ± 1.3	NS	8.7 ± 0.5	NS
MC 903	10.0	41.4 ± 3.8	NS	10.0 ± 0.2	NS
MC 903	100.0	36.4 ± 1.5	<0.05	8.4 ± 0.5	NS

Female Lewis rats were treated with $1,25(OH)_2D_3$ (0.5 μg/kg/day), $1\alpha(OH)D_3$ (1.0 μg/kg/day) or MC 903 (1, 10 or 100 μg/kg/day) i.p. for 7 days. The tibiae were removed and the metaphyseal bone was cut, dried and weighed. Calcium content of the metaphyses was determined after washing of the bone. Results were expressed as mg dry weight or mg calcium per metaphysis (N = 4).

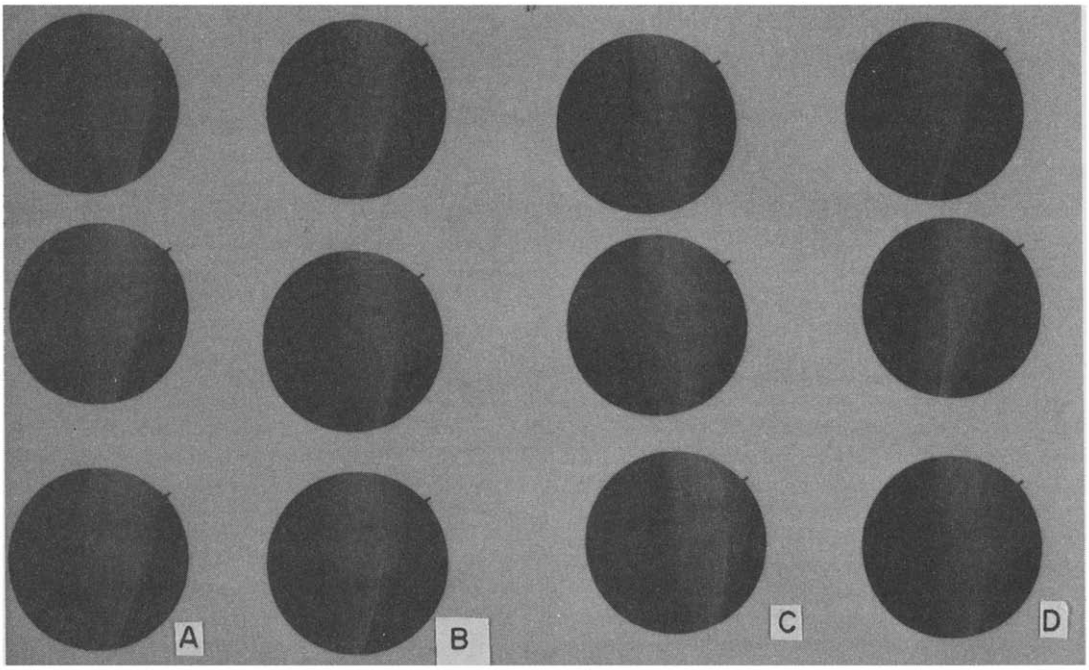

Fig. 3. Rachitic rats treated with MC 903 and $1,25(OH)_2D_3$. Radiograms of proximal tibia/distal femur (3 rats per group): (A) untreated control; (B) MC 903 100 μg/kg p.o.; (C) $1,25(OH)_2D_3$ 0.5 μg/kg p.o.; (D) $1,25(OH)_2D_3$ 0.05 μg/kg p.o.

on rachitic bone. It may be concluded that the vitamin D activity of MC 903 in this model is at least 100–200 times less than that of $1,25(OH)_2D_3$.

DISCUSSION

The recent findings of specific receptors for $1,25(OH)_2D_3$ in a number of malignant cells lines [8], normal monocytes and macrophages [24, 25], activated T-lymphocytes [26], normal keratinocytes and dermal fibroblasts [6, 27] and the associated effects on cell proliferation and cell differentiation have raised the possibility that this vitamin D metabolite is involve in a number of regulatory biological effects in addition to those related to calcium and phosphate metabolism. Vitamin D metabolites may thus be of potential interest in the treatment of a number of proliferative diseases, but their useful therapeutic range is limited by their potent effects on calcium metabolism, leading to hypercalcemia, hypercalciuria and calcifications in kidney, heart and blood vessels.

These considerations have stimulated our interest in the development of new vitamin D derivatives and analogues which have retained their regulatory effects on cell differentiation and proliferation, but which exhibit decreased activity on calcium metabolism. The present report deals with the effects of a new vitamin D analogue MC 903, which shows a promising profile in this respect.

Our investigations show that MC 903 is very similar to the natural metabolite $1,25(OH)_2D_3$ itself, when tested in a culture system of human histiocytic lymphoma cells with high affinity receptors for $1,25(OH)_2D_3$ [18]. MC 903 was shown to displace [^{3}H]-labelled $1,25(OH)_2D_3$ from the cytoplasmic receptor at least as efficiently as $1,25(OH)_2D_3$. Direct binding studies with MC 903 have not yet been performed due to the lack of radiolabelled MC 903 with the required specific activity. MC 903 induced cell differentiation and inhibited cell proliferation and DNA-synthesis at concentrations comparable to those of $1,25(OH)_2D_3$. The high specificity of the cellular receptor for MC 903 and $1,25(OH)_2D_3$ was demonstrated by the fact that the closely related analogue $1\alpha(OH)D_3$ was more than 10,000 times less efficient than MC 903 or $1,25(OH)_2D_3$ in the displacement of [^{3}H]-$1,25(OH)_2D_3$ from the cells. $1\alpha(OH)D_3$ requires metabolic conversion to $1,25(OH)_2D_3$ before being able to bind to the receptor. In the assays of cell differentiation and cell proliferation, where the compounds were present for 4 days, $1\alpha(OH)D_3$ was approximately 100 times less active than MC 903 and $1,25(OH)_2D_3$. The reason for these differences in $1\alpha(OH)D_3$ activity is not known, but the possibility exists that the U 937 cells may contain a hydroxylase capable of mediating the metabolic conversion of $1\alpha(OH)D_3$ to $1,25(OH)_2D_3$. In this respect it is known that normal macrophages possess 1α-hydroxylase activity [28].

When MC 903 was tested for its effects on calcium metabolism in rats in comparison with $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$, it was found that MC 903 was 100–200 times less potent than the 2 reference compounds. $1,25(OH)_2D_3$ at 0.5 μg/kg/day and $1\alpha(OH)D_3$ at 1.0 μg/kg/day strongly increased urinary calcium excretion, serum calcium levels and calcium mobilisation from bone. Comparable effects were observed with MC 903 at 100 μg/kg/day. The

compounds were administered both orally and intraperitoneally in order to avoid errors due to differences in absorption. Later experiments have shown that MC 903 is well absorbed after p.o. administration (personal communication E. Eilertsen). Further proof of the low vitamin D activity of MC 903 was obtained by administration to rachitic rats and observation of the low healing effect on the calcification defects in bone.

In view of its strong direct effects on cell differentiation and on cell proliferation, the low vitamin D activity of MC 903 is surprising. A number of explanations are presently under investigation. MC 903 may exhibit a decreased ability to bind to the intestinal receptor that mediates calcium absorption from the intestine or, once bound, MC 903 may not be able to activate the transfer of the cytoplasmic receptor to the nucleus and to induce DNA-transcription. To elucidate this question quantitative binding studies with the isolated intestinal receptor, as well as studies on calcium transport *in vitro*, using the everted gut-sac technique [29], are indicated. Alternatively, MC 903 may be degraded more rapidly in the intestinal cells than in the U 937 cells. Another possibility is increased metabolic degradation of MC 903 in the liver or defective transport to target organs. Transport of vitamin D metabolites in the blood is mainly performed by binding to a specific plasma protein, the vitamin D binding protein [30]. The functional role of this protein seems to be protection of labile metabolites, transport of poorly soluble compounds and delivery to the appropriate tissues. Studies are presently under way to determine the role of the vitamin D binding protein in the transport and metabolism of MC 903.

The outcome of these studies will determine the role of MC 903 as a possible candidate for systemic treatment of various proliferative disorders. Meanwhile, MC 903 appears as a promising candidate for topical treatment of psoriasis, in so far as encouraging clinical results have already been shown in this disease after administration of classical vitamin D metabolites [14, 15]. MC 903 is currently under clinical evaluation in patients with psoriasis.

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