Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Monotherapy with the vitamin D_3 analogue MC1288 does not result in prolonged kidney allograft survival in rhesus monkeys

Michel Vierboom,[1] C. Johnsson,[2] Bert 't Hart[1] and Margreet Jonker[1]

1 Department of Immunobiology, Biomedical Primate Research Centre, Rijswijk, The Netherlands
2 Department of Transplantation Surgery, University Hospital, Uppsala, Sweden

Keywords
immunosuppression, kidney transplantation, vitamin D.

Correspondence
Michel P.M. Vierboom PhD, Department of Immunobiology; Division Immunotherapy, Biomedical Primate Research Centre, PO Box 3306, 2280 GH Rijswijk, The Netherlands.
Tel.: +31 15 284 2654; fax: +31 15 284 3999; e-mail: vierboom@bprc.nl

Received: 13 October 2005
Revision requested: 4 November 2005
Accepted: 3 February 2006

doi:10.1111/j.1432-2277.2006.00299.x

Summary

The active form of vitamin D3, $1,25(OH)_2D_3$, has pronounced immunoregulatory properties and is a potential treatment of immune-based disorders. However, the central role of this hormone in calcium and bone metabolism complicates its long-term use as an immunomodulator. Some newly developed vitamin D3-derived analogues, such as MC1288, have an improved immunoregulatory potential and prolong allograft survival in rodent models. Such compounds might be a valuable component of immunosuppressive treatment regimen in transplantation and autoimmunity. The rhesus monkey provides a useful model for the preclinical validation of new therapeutic strategies for transplantation. The present study shows that MC1288 inhibits both proliferation and interferon-γ production by rhesus peripheral blood mononuclear cells in a mixed lymphocyte reaction. We have tested the maximum tolerated dose of MC1288 in a rhesus monkey model of kidney transplantation. The observed effects on serum calcium and parathyroid hormone confirm the *in vivo* activity of MC1288. However, as a monotherapy, MC1288 did not cause prolongation of the kidney allograft survival in rhesus monkeys.

Introduction

Current immunosuppressive treatment regimens in kidney transplantation have substantially improved short-term allograft survival. However, the induction of long-term stable renal allograft survival is limited by chronic allograft nephropathy (CAN), involving both immunological and nonimmunological processes. Moreover, the long-term use of immunosuppressive drugs is accompanied with severe side effects including toxicity [1], increased risk of infection [2] and a higher incidence of some cancers [3]. Immunomodulating drugs with fewer side effects are urgently needed.

The active form of vitamin D_3 ($vitD_3$), $1,25(OH)_2D_3$, has pronounced immunoregulatory properties in rodents [4, 5]. It binds with high affinity to the vitamin D_3 receptor (VDR) and is a potent inhibitor of T lymphocyte activation [6, 7]. Furthermore, it prevents the differentiation and maturation of dendritic cells (DCs) [8, 9] creating a tolerogenic environment.

The use of $vitD_3$ for chronic treatment of immune-based disorders (autoimmunity, transplantation) is complicated by its calcium mobilizing effect. $VitD_3$ is part of a regulatory feedback loop to secure blood Ca^{2+} homeostasis [10]. Other members of this control mechanism are parathyroid hormone (PTH) and calcitonin. Low blood Ca^{2+} concentrations stimulate PTH production by the parathyroid gland, which promotes Ca^{2+} release from the bone, Ca^{2+} uptake in the intestine and Ca^{2+} resorption by the kidney. These processes result in an increased blood Ca^{2+} concentration. When the Ca^{2+} level is too high the thyroid gland is stimulated to produce calcitonin which induces the Ca^{2+} deposition in the bone and reduces the Ca^{2+} uptake in the intestine and kidney. Moreover, PTH

promotes production of the active form of vitamin D_3, $1,25(OH)_2D_3$ in the kidney, which in turn stimulates Ca^{2+} resorption in the intestine. Administration of $1,25(OH)_2D_3$ in doses required for immune modulation resulted in hypercalceamia [11]. New vitD_3 analogues have been developed with either reduced calcium mobilizing potential or more potent immunoregulatory capacity. The availability of these analogues has renewed interest in their use as immunomodulatory agents in transplantation therapy [4] and autoimmunity [5].

MC1288 is such an analogue [12]. Although its calcium mobilizing capacity in rats equals that of $1,25(OH)_2D_3$. It has a 100–200-fold more potent immunosuppressive effect *in vitro* [12]. In rats, the use of MC1288 as a monotherapy has resulted in prolonged aortic [13], cardiac [14], small bowel [14], kidney [13] and pancreatic islets allograft survival. Furthermore, treatment with MC1288 in combination with low dose of cyclosporine A (CsA) in rats with aortic allografts resulted in the reduction of chronic rejection [15, 16].

The validity of some organ transplantation models may be questioned, because many new therapies that suppressed graft rejection in rodents were subsequently shown to be ineffective in human patients [17, 18]. Non-human primates are often used in transplantation research to validate therapeutic effects observed in rodents before they are tested in patients. Before considering the future inclusion of MC1288 in a combination therapy, we have tested the effect of MC1288 as a monotherapy at the maximum tolerated dose on allograft survival in a rhesus monkey model of kidney transplantation. Our results show no beneficial effect of MC1288 on the survival of the allograft.

Material and methods

Animals

Fourteen rhesus monkeys (*Macaca mulatta*) between 4 and 10 years of age were obtained from the colony at the Biomedical Primate Research Centre (Rijswijk, The Netherlands). Male and female animals were used. All animals were typed for Mamu-A, B and DR antigens by serology. All donor-recipient combinations were mismatched for at least one class-I allele (A or B) and one class-II allele (DR). The recipient–donor pairs were compatible for ABO antigens. Pairing of mismatched donors and recipients was confirmed by a positive unidirectional mixed lymphocyte response (MLR; mean stimulation index ≥12.0). Recipients had no history of allogeneic immunization through blood transfusion, transplantation or pregnancy.

The animals were declared in good health by the veterinarian staff and showed normal values for hematology and clinical chemistry before being entered into the experiment. During the experiment, the animals were individually housed to allow for the evaluation of urine production. The ambient room temperature was maintained between 21 ° and 23 °C. During the study, the monkeys received a daily diet consisting of AM-II food pellets (Hope Farms, Woerden, The Netherlands), enriched with fruit and bread. Drinking water was available *ad libitum*. For routine handling, animals were sedated with ketamine (10 mg/kg). All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee installed by Dutch law.

In vitro analysis

Mixed lymphocyte response

The inhibitory potential of the vitD_3 analogue MC1288 on alloantigen-specific proliferation was tested *in vitro* through a primary unidirectional MLR by co-culturing peripheral blood mononuclear cells (PBMC) from genetically unrelated rhesus monkeys. PBMC, from MHC-DR mismatched rhesus monkeys, were isolated from heparinized venous blood by density gradient centrifugation using Lymphocyte Separation Medium (LSM; ICN Biomedical, Aurora, OH, USA). Subsequently, 10^5 responder rhesus monkey PBMC were seeded into 96-well round-bottom plates with 10^5 allogeneic irradiated (30 Gy) stimulator rhesus monkey PBMC for 5 days in the presence of titrated amounts of MC1288 (see Fig. 1a). MLRs were performed in RPMI-medium (HEPES-buffered) supplemented with 10% fetal calf serum (FCS), 2mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 2×10^{-5} M 2-ME (all obtained from GIBCO-Invitrogen, Breda, The Netherlands) at 37 °C in humidified air containing 5% CO_2. After 5 days radiolabeled thymidine (^{3}H-thymidine; 1 μCi/well; GE Healthcare Europe, Munich, Germany) was added, and the incorporation into the DNA of proliferating cells was determined after 18 h incubation using a Matrix 9600 beta counter (Packard Instruments, Meridan, CT, USA). All tests were performed in triplicate. The proliferation is expressed as counts per minute (CPM).

ELISA for rhesus monkey interferon-γ

ELISA kits [Cytokine ELISA/human interferon-γ (IFN-γ), U-CyTech BV, Utrecht, The Netherlands] for the detection of rhesus monkey IFN-γ were used according to the manufacturer's instructions.

Pharmacokinetic study determining the tolerability of MC1288

MC1288 was dissolved in a vehicle solution at a concentration of 25 μg/ml (Batch no.: 95-327-22; Leo Pharma-

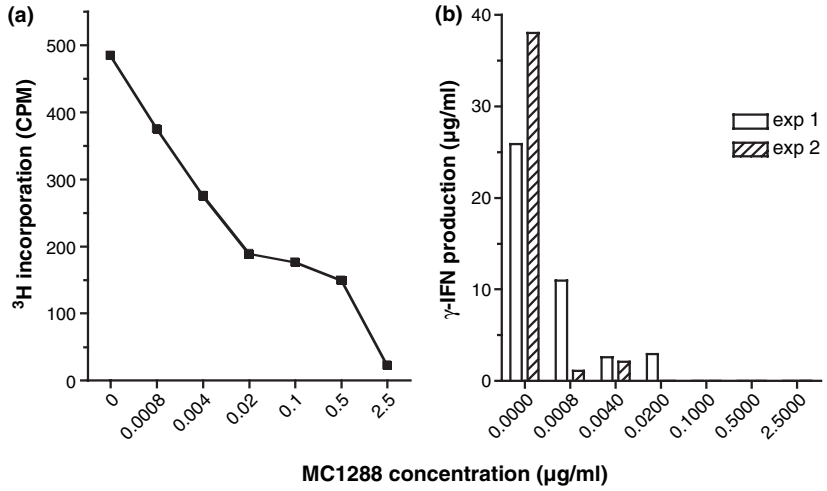

Figure 1 MC1288 inhibits *in vitro* alloresponses. Both the proliferative response (a) and the IFN-γ production (b) are suppressed in the presence of MC1288.

ceutical Products, Ballerup, Denmark). The material was stored at 4 °C and adjusted to the calculated dose with vehicle solution immediately before administration.

Six animals were used to determine the dose of MC1288 to be used in the transplantation study. First, two animals were given a low dose (0.06 μg/kg/day) for the first week. The dose was doubled for weeks 2–4. No effect on serum Ca^{2+} concentration was observed. Subsequently, two more animals were given a higher dose (0.8 μg/kg/day) during all 4 weeks. A third group was started on 0.3 μg/kg/day for the first 4 days, increased to 0.6 μg/kg/day for days 5–11 and then 1.2 μg/kg/day until day 28. Dosing levels were adjusted based on calcium levels at day 4 and 10. Dosing was adjusted for both animals in one group simultaneously. Serum was taken on days −14, −7, −5, 0, 4, 10, 16, 23, 29 for Ca^{2+} determination.

The animals were initially adapted to the dosing procedure by gastric gavage by dosing them with 1 ml/kg vehicle solution (volume used for the transplantation study) for 7 days. All animals were dosed daily.

Efficacy of MC1288 in kidney transplantation

Eight animals were used in the actual transplantation study. These animals were divided into two groups: four were treated with the maximal tolerable dose and four were controls and treated with vehicle solution only (see Tables 1 and 2). Dosing started 3 days before the transplantation and continued until rejection.

Heterotopic kidney allotransplantation with bilateral recipient nephrectomy was performed as described with some modification [19]. General anesthesia during surgery consisted of O_2/N_2O/isoflurane. In brief, the donor kidney was perfused with University of Wisconsin preservation fluid and kept on ice until they were transplanted. The cold ischemia time was <3 h. The post-transplant

Table 1. Dosing periods and dosing amounts of animals in the pharmacokinetic/tolerability study.

Group number	Treatment	Dose period (days)	MC1288 dose (μg/kg/day)	Animal ID
1	Low dose	0–7	0.06	4096, 8927
		8–28	0.12	
2	Medium dose	0–28	0.8	8925, 8987
3	High dose	0–4	0.3	8961, 4119
		5–11	0.6	
		12–28	1.2	

prophylactic treatment of the recipients included antibiotics (Synulox®; Pfizer, Capellle a/d IJssel, The Netherlands), analgesics (Temgesic®; Schering-Plough BV, Maarssen, The Netherlands) and subcutaneous fluids (100 ml of 0.45% NaCl + 2.5% glucose) for 5 days.

The clinical condition of the animals was monitored by daily visual inspection and biweekly hematological and clinical chemistry blood values. Serum urea levels were monitored by blood dipstick analysis (Merckognost ureum no. 11001, Merck, NJ, USA). If serum urea increased above 20%, blood was collected to determine serum creatinin. Rejection of the kidney was defined as an increase of serum creatinin over 700 μmol/l. A rejection episode was not treated and the animals were sacrificed and necropsy performed.

Results

MC1288 inhibits alloresponses by rhesus monkey PBMC

Peripheral blood mononuclear cells from two rhesus monkeys (AVD, BB26) were co-cultured in the presence of titrated amounts of MC1288. The vitD$_3$ analogue was found to inhibit both chosen parameters of allostimulation, i.e. proliferation (Fig. 1a) and IFN-γ production

Table 2. Dosing regimen and dosing amounts of animals used in the transplantation study.

Group number	Treatment	Dose period (days)*	MC1288 dose (μg/kg/day)	Animal ID		Graft survival (days)
				Recipient	Donor	
4a	Low dose	Day −3 to day 3	1	25A	BB69	7
		Day 4	0.75	4134	BB69	8
		Day 5	1.5			
		Day 6 to rejection	1			
4b	Medium dose	Day −3 to day −2	1	1XF	BB73	6
		Day −1 to rejection	2	8938	BB73	7
5	No treatment	Day −3 to rejection	Vehicle only	8790	8927†	6
				4129	8927†	6
				8915	8987†	7
				4138	8987†	5

*The days indicated are relative to the day of transplantation.
†These monkeys were used as kidney donor following a MC1288 wash out of >6 months after the tolerability study.

(Fig. 1b) of the allogeneic cultures in a dose dependent fashion and at low concentrations of 4–20 ng/ml. The viability of the cultures at these concentrations was comparable with uninhibited control cultures as determined with trypan bleu (data not shown).

Calcium mobilization by MC1288

First, the maximum tolerated dose of MC1288 was determined. This parameter was defined as the highest dose that caused a marginal increase of the serum Ca^{2+}-level during repeated daily oral administration for 28 days. Three groups of two animals (group 1, 2 and 3; Table 1) were tested. Serum was collected at different time points.

Animals in group 1 (animal ID: 4096 and 8927) first received a low dose of MC1288 (0.06 μg/kg/day) for 7 days. When calcium levels stabilized the dose of MC1288 was increased (0.12 μg/kg/day) from day 8–28. No effect on the serum Ca^{2+} concentration was observed in either animal (Fig. 2a). Animals in group 2 (animal ID: 8987 and 8925) were treated with an intermediate dose (0.8 μg/kg/day) of MC1288 for 28 days. Except for one higher Ca^{2+} concentration observed in animal 8987 on day 23, stable levels of Ca^{2+} were observed in both animals (Fig. 2b) (Ca^{2+} increase <10%) in the serum. Both animals in group 3 (animal ID: 4119 and 8961) demonstrated a marked increase (>20%) in the serum Ca^{2+} concentration when the dose of MC1288 was increased from 0.6 to 1.2 μg/kg/day on day 12 (Fig. 2c). The maximum tolerated concentration of MC1288 administered was determined to be in between 0.8 and 1.2 μg/kg. It was, therefore, decided to use a starting dose of 1 μg/kg/day of MC1288 in the transplantation study. Note that this dose is 10-fold higher than the tolerated concentration in rats [16, 20].

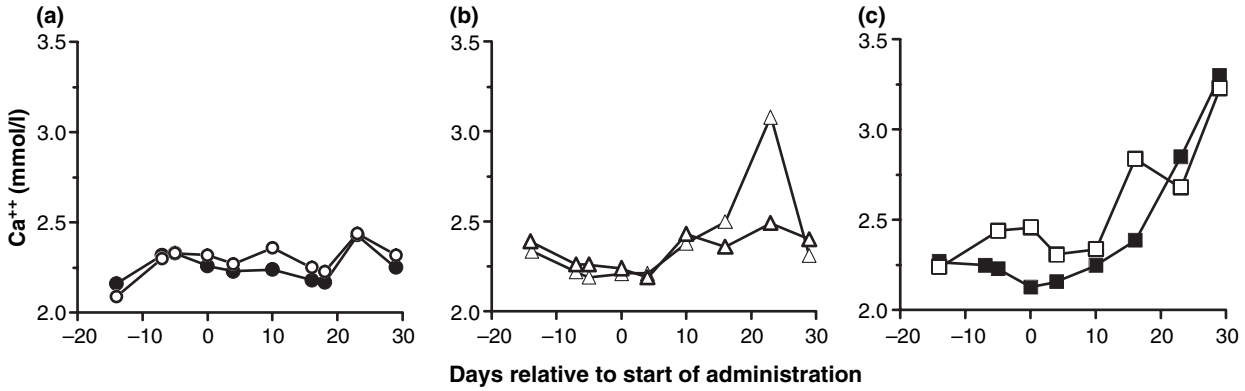

Figure 2 MC1288 affect serum calcium levels (mmol/l) at high doses. MC1288 was administered at different doses to determine the maximum tolerated dose. (a) Group 1 (8927, ○; 4096, ●) received a low dose of MC1288 (day 0–7: 0.06 μg/kg; day 8–28: 0.12 μg/kg); (b) group 2 (8925, △; 8987, ▲) received an intermediate dose of MC1288 (day 0–28: 0,8 μg/kg); (c) group 3 (8961, □; 4119, ■) received a high dose (day 0–5: 0.3 μg/kg; day 6–11: 0.6 μg/kg; day 12–28: 1,2 μg/kg).

Transplantation experiment

Before a combination of a new drug with current treatments can be considered its effect needs to be evaluated as a single therapy. The efficacy of MC1288 as administered as a monotherapy was tested in a kidney transplantation experiment in rhesus monkeys. Previously, treatment with monotherapy of MC1288 in rodent allograft models resulted in prolonged allograft survival.

One group of four animals was transplanted and treated with vehicle solution only (group 5). The second group of four animals was transplanted and treated with MC1288. Animals in group 4a received ±1 μg/kg/day while the 2 animals in group 4b received ±2 μg/kg/day during the period they were transplanted (see Table 2). Serum was collected to establish the Ca^{2+} mobilization by MC1288 and PTH-levels. Furthermore, we determined serum creatinin levels to determine rejection of the kidney allograft.

Effect of MC1288 on serum Ca^{2+} and PTH

MC1288 induced a marked increase in Ca^{2+} blood levels in three animals (Fig. 3a; animal ID: 1XF, 8938 and 4134) during the course of treatment. The Ca^{2+} increase was associated in a sharp decrease (fivefold) of PTH production (Fig. 4a). One animal showed an opposite response to the MC1288 treatment, namely a decreased Ca^{2+} level (Fig. 3a; animal ID: 25A) with a markedly increased PTH production (Fig. 4a). Three of four animals in the placebo-treated group (Fig. 3b; animal ID: 4129, 8915 and 4138) showed a decrease in Ca^{2+} blood levels, especially after the kidney transplantation. This was associated with a mean fivefold increase of PTH. One animal in this group showed an increase of the Ca^{2+} level at the day of rejection compared with the previous value (Fig. 3b: open circle; animal ID: 8790). This animal showed a twofold reduction of PTH production (Fig. 4b). As a general trend, increased serum MC1288 level were found associated with decreased serum PTH levels; an observation also made in humans treated with 1,25$(OH)_2D_3$ [21].

Effect of the maximum tolerated MC1288 dose on graft survival

The kidney graft survival was measured from the day of transplantation to the first rejection episode. Rejection was determined by an increase of serum creatinin over 700 μmol/l. During rejection episodes the animals did not receive any treatment in addition to MC1288. We observed only a minor difference in serum creatinin levels between untreated animals or rhesus monkeys treated with MC1288 (Fig. 5). MC1288 had no detectable suppressive effect on the rejection of the kidney allograft as the mean survival time of animals receiving only vehicle was 6 ± 0.8 days ($n = 4$), whereas the mean survival time of animals receiving MC1288 was 7 ± 0.8 ($n = 4$). Rejection data for individual animals are given in Table 2. Rejection was confirmed by histology.

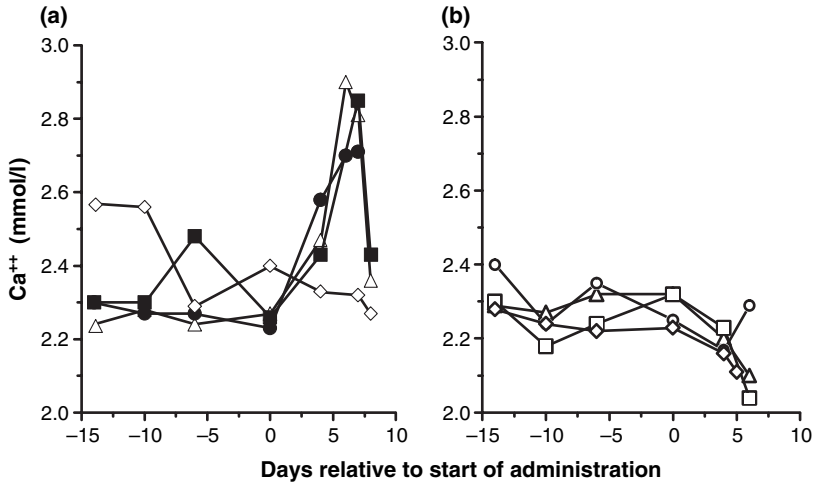

Figure 3 The effect of MC1288 on serum calcium levels (mmol/l) of kidney transplanted rhesus monkeys before and after transplantation. (a) Daily treatment with MC1288 started at day –3 and continued until day 28 unless animals were sacrificed earlier for ethical reasons. This resulted in a marked increase of the Ca^{2+} serum levels in three of four animals (1XF, ●; 8938, ▲; 4134, ■) at the time of rejection and one animal showing a decrease (25A, ◆). Two animals (25A, 4134) received 1 μg/kg/day and two animals (1XF, 8938) received 2 μg/kg/day. (b) The placebo-treated group only received vehicle-solution and followed the same treatment regimen as the MC1288 treated group. Three animals showed a marginal decrease in Ca^{2+} at the time of rejection (4129, △; 8915, □; 4138, ○) and 1 animal a minor increase (8790, ◇).

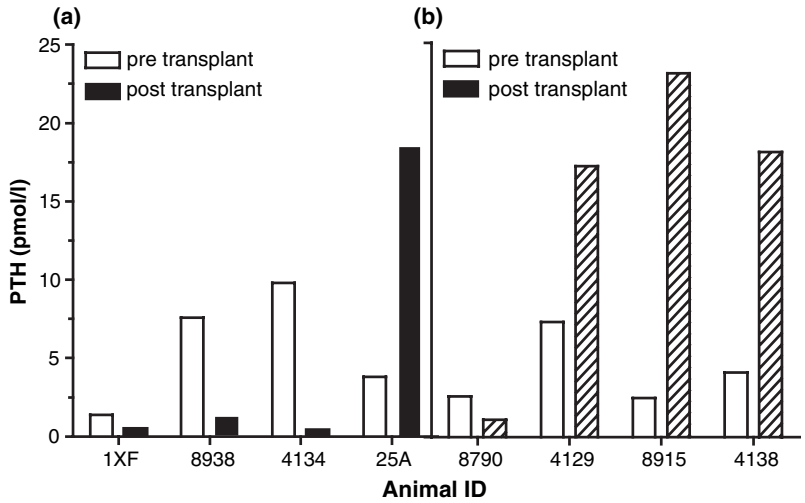

Figure 4 Treatment with MC1288 results in a depression of serum PTH-levels. (a) Three of four animals treated with MC1288 showed a marked decrease in serum PTH levels at the day of rejection [1XF (d6); 8938 (d7); 4134 (d8)] compared with pretransplant PTH levels [1XF (d-14); 8938 (d-14); 4134 (d-5)]. Animal 25A showed a marked increase in serum PTH levels at the day of rejection (d7) compared with pretransplant PTH level (d-5). (b) Three of four placebo-treated animals showed a marked increase in serum PTH levels at the day of rejection [4129 (d6); 8915 (d7); 4138 (d5)] compared with pretransplant PTH levels [4129 (d-10); 8915 (d-21); 4138 (d-21)]. Animal 8790 showed a decrease in serum PTH levels at the day of rejection (d6) compared with pretransplant PTH level (d-10). The day of PTH level measurement for each animal is indicated between the brackets.

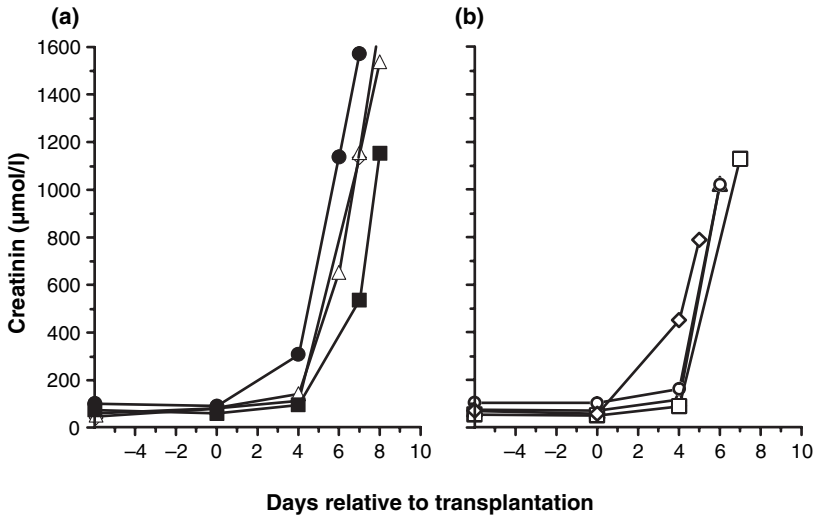

Figure 5 Treatment with MC1288 does not prolong graft survival. An increase serum creatinin levels is indicative for kidney graft rejection. (a) Animals treated with MC1288 daily (1XF, ●; 8938, ▲; 4134, ■; 25A, ◆) all show a marked increase in creatinin after day 4. (b) This is comparable with the increase of serum levels of creatinin after transplantation in the placebo-treated group (4129, △; 8915, □; 4138, ◇; 8790, ○).

Discussion

We have tested the effect of the new vitamin D3 analogue MC1288 in a well-established rhesus monkey model of kidney allograft survival. MC1288 differs from the natural hormone 1,25(OH)$_2$D$_3$ in its stereochemistry at the C$_{20}$ position. This modification leaves high affinity binding to the vitamin D3 receptor (VDR) intact and improves the immunosuppressive capacity *in vitro* by a factor of 100 compared with 1,25(OH)$_2$D$_3$ [12]. Despite the improved immunosuppressive effect, and a significant suppressive effect on the *in vitro* alloresponse of rhesus monkey T-cells, our results show no effect of MC1288 on the survival of a kidney allograft in rhesus monkeys. This is in marked contrast to the positive effect of MC1288 in rat allograft models.

MC1288 was found to inhibit T-cell proliferation and IFN-γ production at a dose of 4–20 ng/ml in a one-way MLR. Similar *in vitro* suppressive effects have been observed on rodent lymphocytes [12]. We then tested

MC1288 in a kidney allograft survival model in rhesus monkeys at the maximum tolerated dose that was determined on the calcium mobilization effect *in vivo*. Ca^{2+} mobilization appeared most prominent at a concentration of 1.2 μg/kg but changed marginally at a concentration of 0.8 μg/kg. The chosen dose of 1 μg/kg for the transplantation experiment was 10–100-fold higher than the tolerated dose in rats [13, 16]. The higher end organ tolerance of rhesus monkeys compared with rats stresses the importance of vitD3 testing in nonhuman primates. The observed effects of MC1288 in the rhesus monkeys were in the anticipated range on the basis of literature data, indicating that the administered MC1288 dose was effective in this species [22]. Predictably, low Ca^{2+} level observed in monkeys 4129, 8915, 4138 and 25A (last value in Fig. 2) corresponded with a high PTH concentration (see Fig. 3). Conversely, the increased amounts of Ca^{2+} in several animals (1XF, 8938, 4134 and 8790; Fig. 2) corresponded with a lower PTH serum concentration (Fig. 3). A specific complication in kidney transplantation is that the therapeutic compound directly affects impact on Ca^{2+} homeostasis and thus influence the proper functioning of the organ. The feedback system may add to this as the vitD$_3$ analogue can inhibit the binding of autologous vitD3 by the organ itself.

The tested vitD$_3$ analogue might still be effective in combination with other immunosuppressive drugs, such as cyclosporine, for example to lower the dose and reduce nephrotoxicity [15, 20]. Its potential to prevent CAN in rats would warrant further investigation into combination strategies [23]. Furthermore, the immunomodulatory effect on DC might promote the induction of regulatory T cells [24], which in turn can mediate transplant tolerance [25].

In conclusion, the vitD3 analogue MC1288 fails to reproduce the promising effects observed in a variety of rat transplantation models [13–16] when tested in a valid preclinical model of kidney allograft survival in nonhuman primates. We regard it important to publish negative results, especially when discrepant effects have been observed between rodents and nonhuman primates [26]. The path of immunotherapy development for immune-based disorders is paved with failed and interrupted trials, not only in transplantation [17], but also in autoimmunity, multiple sclerosis for example [27]. Failed trials should not be ignored but should be used to improve the quality of preclinical research, in particular the development of better predictive animal models [18].

Acknowledgments

The authors thank Dr Miriam Ossevoort, Jan Ringers (MD) and Ella Sick for their expert technical assistance and contributions. This research was in part supported by a grant from the EU (BMH4-CT96–0127) and Leo Pharmaceuticals.

References

1. Denton MD, Magee CC, Sayegh MH. Immunosuppressive strategies in transplantation. *Lancet* 1999; **353**: 1083.
2. Fishman JA, Rubin RH. Infection in organ-transplant recipients. *N Engl J Med* 1998; **338**: 1741.
3. Penn I. Post-transplant malignancy: the role of immunosuppression. *Drug Saf* 2000; **23**: 101.
4. Adorini L. 1,25-Dihydroxyvitamin D3 analogs as potential therapies in transplantation. *Curr Opin Investig Drugs* 2002 Oct; **3**: 1458.
5. Adorini L. Immunomodulatory effects of vitamin D receptor ligands in autoimmune diseases. *Int Immunopharmacol* 2002 Jun; **2**: 1017.
6. Lemire JM, Adams JS, Kermani-Arab V, Bakke AC, Sakai R, Jordan SC. 1,25-Dihydroxyvitamin D3 suppresses human T helper/inducer lymphocyte activity *in vitro*. *J Immunol* 1985 May; **134**: 3032.
7. Bhalla AK, Amento EP, Serog B, Glimcher LH. 1,25-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation. *J Immunol* 1984; **133**: 1748.
8. Penna G, Adorini L. 1α,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol* 2000; **164**: 2405.
9. Piemonti L, Monti P, Sironi M, Fraticelli P, Leone BE, Dal Cin E, *et al.* Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. *J Immunol* 2000 May 1; **164**: 4443.
10. Suki WN, Rose D. Renal transport of calcium, magnesium and phosphate. In: Brenner BM, ed. *The Kidney*. Philadelphia: Saunders, 1996: 472.
11. Brown AJ. Therapeutic uses of vitamin D analogues. *Am J Kidney Dis* 2001 Nov; **38**(5 Suppl 5): S3.
12. Binderup L, Latini S, Binderup E, Bretting C, Calverley M, Hansen K. 20-epi-vitamin D3 analogues: a novel class of potent regulators of cell growth and immune responses. *Biochem Pharmacol* 1991; **42**: 1569.
13. Kallio E, Hayry P, Pakkala S. MC1288, a vitamin D analogue, reduces short- and long-term renal allograft rejection in the rat. *Transplant Proc* 1996 Dec; **28**: 3113.
14. Johnsson C, Tufveson G. MC1288 - a vitamin D analogue with immunosuppressive effects on heart and small bowel grafts. *Transpl Int* 1994; **7**: 392.
15. Raisanen-Sokolowski AK, Pakkala IS, Samila SP, Binderup L, Hayry PJ, Pakkala ST. A vitamin D analog, MC1288, inhibits adventitial inflammation and suppresses intimal lesions in rat aortic allografts. *Transplantation* 1997 Apr 15; **63**: 936.
16. Raisanen-Sokolowski A, Pakkala I, Vuoristo P, Samila S, Binderup L, Hayry P, *et al.* Effect of MC1288, a vitamin D

analogue, on lymphocyte proliferation *in vitro* and rat aortic allograft arteriosclerosis *in vivo*. *Transplant Proc* 1996 Apr; **28**: 919.
17. Sachs DH. Tolerance: of mice and men. *J Clin Invest* 2003 Jun; **111**: 1819.
18. 't Hart B, Amor S, Jonker M. Evaluating the validity of animal models for research into therapies for immune-based disorders. *Drug Discov Today* 2004 Jun; **9**: 517.
19. Neuhaus P, Neuhaus R, Wiersema HD, Borleffs JC, Balner H. The technique of kidney transplantation in rhesus monkeys. *J Med Primatol* 1982; **11**: 155.
20. Johnsson C, Binderup L, Tufveson G. The effects of combined treatment with the novel vitamin D analogue MC1288 and cyclosporine A on cardiac allograft survival. *Transpl Immunol* 1995 Sep; **3**: 245.
21. Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, *et al.* Hypovitaminosis D in medical inpatients. *N Engl J Med* 1998; **338**: 777.
22. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004 Dec; **80**: 1678S.
23. Hullett DA, Laeseke PF, Malin G, Nessel R, Sollinger HW, Becker BN. Prevention of chronic allograft nephropathy with vitamin D. *Transpl Int* 2005 Oct; **18**: 1175.
24. Gregori S, Casorati M, Amuchastegui S, Smiroldo S, Davalli AM, Adorini L. Regulatory T cells induced by 1 alpha,25-dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. *J Immunol* 2001 Aug 15; **167**: 1945.
25. Graca L, Thompson S, Lin CY, Adams E, Cobbold SP, Waldmann H. Both CD4(+)CD25(+) and CD4(+)CD25(−) regulatory cells mediate dominant transplantation tolerance. *J Immunol* 2002; **168**: 5558.
26. Steinman RM, Mellman I. Immunotherapy: bewitched, bothered, and bewildered no more. *Science* 2004 Jul 9; **305**: 197.
27. Wiendl H, Hohlfeld R. Therapeutic approaches in multiple sclerosis: lessons from failed and interrupted treatment trials. *Bio Drugs* 2002; **16**: 183.

