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Pharmacokinetics of cyclosporine in monkeys after oral and intramuscular administration: relation to efficacy in kidney allografting

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Abstract In cynomolgus and rhesus monkeys, the dose-normalized exposure of cyclosporine administered orally as microemulsion preconcentrate (Neoral) was lower than that upon intramuscular administration. For oral administration, mean values (± SD) of C_{max}, 24-h area-under-the curve (AUC) and 24-h trough level, all normalized for a 1 mg/kg dose, were 20 ± 9 ng kg/mg ml, 210 ± 70 ng h kg/mg ml and 2.6 ± 0.9 ng kg/mg ml, respectively. For intramuscular administration, levels were about 5.5-fold, 9-fold and 22-fold higher. Based on pharmacokinetic data, the efficacy of oral cyclosporine treatment (without any other immunosuppressant) was evaluated in life-supporting cynomolgus monkey kidney allotransplantation. Rejection-free kidney allograft survival could be achieved using oral cyclosporine monotherapy with average 24-h trough concentrations above 100 ng/ml during maintenance treatment. Typically, daily oral doses of 100 mg/kg–150 mg/kg during the first two weeks post-transplantation, followed by daily 30 mg/kg–100 mg/kg dose levels during subsequent maintenance can result in long-term allograft survival, with 24-h average trough levels in individual animals during maintenance between 110 ng/ml and 700 ng/ml.

Keywords Cyclosporine · Cynomolgus monkey · Kidney transplantation · Neoral · Pharmacokinetics

Abbreviations *AUC* Area-under-the-curve · *BPRC* Biomedical Primate Research Centre, Rijswijk, The Netherlands · *MLR* Mixed lymphocyte reactivity

Introduction

Cyclosporine is an effective immunosuppressive that nowadays forms a basic component of immunosuppressive protocols in clinical transplantation. The compound formulated as microemulsion preconcentrate (Neoral, registered trade mark of Novartis Pharma Basel, Switzerland) has been successfully introduced as the successor of the compound in oily solution (Sandimmune, registered trade mark of Novartis Pharma Basel, Switzerland). Neoral has the advantage of improved bioavailability, reduced pharmacokinetic variability, and lowered dependence of compound absorption on the physiological state of the gastrointestinal tract [14, 15, 17]. Cyclosporine is furthermore widely used as immunosuppressant in animal models of transplantation, including organ transplantation in non-human primates, in which the compound has so far mostly been administered in oily solution by intramuscular injection. Daily doses of 10 mg/kg–20 mg/kg body weight proved to be sufficient

to reach therapeutic blood concentrations, with trough levels of around 500 ng/ml. At such a dosage, cyclosporine, as single compound or in combination with azathioprine and/or prednisolone, yielded survival of kidney- [2, 13] and liver allografts [28] in rhesus monkeys, and of kidney- [5, 9] and heart allografts [9, 10, 19, 20] in cynomolgus monkeys. In contrast, rejection of a kidney allograft in cynomolgus monkeys has been documented for subtherapeutic intramuscular dosages yielding 24-h trough levels between 40 and 200 ng/ml [30]. In recent years, interest has turned to new aspects of transplantation, and cyclosporine formed a basic immunosuppressant in conditioning regimens to induce chimerism and tolerance in primates [12], and in pig-to-primate xenotransplantation [6, 7, 21, 22, 31, 32]. To mimick a potential clinical application, the compound was administered in a part of these studies orally.

In all studies reported to date there seems to be no difference between cynomolgus- and rhesus monkeys in pharmacokinetics and efficacious dose levels. In contrast, baboons generally require a higher exposure, so that even cyclosporine had to be given at 20–30 mg/kg body weight by intravenous route to induce survival of liver- [16] or heart-lung [4] allografts. Trough levels in the therapeutic range were claimed to be around 2,000 ng/ml in this species. This phenomenon is presumably related to a relative resistance of baboon lymphocytes to cyclosporine-mediated immunosuppression in vitro [27].

Cyclosporine given orally as oily solution is poorly absorbed in primates compared to humans. This has been demonstrated in pharmacokinetic experiments with rhesus monkeys [29] and baboons [26]. We previously reported on the similarly poor absorption of cyclosporine as microemulsion preconcentrate in cynomolgus monkeys. However, with dosages of up to 100–150 mg/kg per day, blood levels in the therapeutic range are obtained [23]. Since pharmacokinetic profiles for intramuscular administration have not been made to date, and since there is a tendency to change from intramuscular to oral administration of cyclosporine in chronic treatment protocols, we initiated a study to compare the pharmacokinetics of cyclosporine administered intramuscularly as oily solution with those of the compound administered orally as microemulsion preconcentrate. These data were compared with the results of transplantation experiments in which monkeys were subjected to life-supporting kidney allografting and treated with cyclosporine as monotherapy. The transplantation studies were performed at the Biomedical Primate Research Centre (BPRC), Rijswijk, The Netherlands, and at Novartis Pharma AG, Basel, Switzerland. Cyclosporine-treated animals in the transplantation studies at BPRC formed part of a study on efficacy of the macrolide immunosuppressant RAD [24].

Materials and methods

Studies at BPRC were performed in accordance to Dutch Animal Welfare Act, and approved by the Institute Animal Use and Care Committee, as required by Dutch law. Studies at Novartis Pharma AG, Basel, Switzerland (Novartis) were performed in accordance with the Swiss Animal Welfare Act dated March 9, 1978, and the accompanying Animal Welfare Regulation of May 28, 1981.

Pharmacokinetic experiment

The studies were performed at BPRC on 10 male cynomolgus monkeys (*Macaca fascicularis*) and 4 male and 4 female rhesus monkeys (*Macaca mulatta*), of about 3–4 years of age and 3–4 kg body weight. The cynomolgus monkeys originated from Mauritius, and the rhesus monkeys were from the colony kept at the BPRC. Under slight ketamine sedation, monkeys received cycloporine at the dose levels indicated below for 4 days each time in the morning. Blood was sampled in EDTA-anticoagulant just before administration on the first, second and third day, and on the fourth day just before administration and at 1, 2, 4, 8, 12, and 24 h after administration. The cynomolgus monkeys received cyclosporine as microemulsion preconcentrate (Neoral drinking solution, 100 mg/ml) by gastric gavage at 2 ml/kg body weight; the administration of the compound was immediately followed by giving water at a volume which was at least twice that of the Neoral administration volume. One group of five animals (C1, C2, C3, C4, C5) received 25 mg/kg body weight, and was subjected to 50 mg/kg 2 weeks later; a second group of five animals (C6, C7, C8, C9, C10) received 100 mg/kg body weight and was subjected to 150 mg/kg 2 weeks later. The rhesus monkeys received cyclosporine in oily solution (Sandimmune drinking solution, 100 mg/ml) intramuscularly. One group of four animals (R1, R2, R3, R4) received a dose of 10 mg/kg body weight, and was subjected to 2.5 mg/kg 2 weeks later; a second group of four animals (R5, R6, R7, R8) received 5 mg/kg body weight and was subjected to 10 mg/kg 2 weeks later.

Kidney transplantation

Heterotopic kidney transplantation was performed in cynomolgus monkeys either at BPRC or at Novartis. Animals were obtained from Mauritius (BPRC, part of the animals at Novartis) or the Philippines (Novartis). Only male animals were used as recipients because in the initiation of these studies technical failures were observed mainly in female recipients. Either male or female animals were used as donors. Most animals were about 3 years of age and had a body weight between 3 and 6 kg. Donor-recipient combinations were selected following different procedures. At BPRC, mismatch for major histocompatibility complex (MHC) antigens was performed by serology using reagents developed for rhesus monkeys [3]: combinations were selected so that there was at least one incompatibility at the DR locus and at least two incompatibilities at the A/B locus. At Novartis, mismatch was evaluated by mixed lymphocyte reactivity (MLR): combinations were selected so that the stimulation index in one-way (host-versus-donor) MLR was at least 10. At both centers, donors and recipients were matched for red blood group antigens (ABO) by using human indicator cells of A or B type (BPRC), or by the saliva inhibition test [8, 25]. Life-supporting heterotopic kidney allografting with bilateral nephrectomy was performed either under inhalation anaesthesia following procedures developed for rhesus monkeys (BPRC) [18] or under intravenous propofol anesthesia (Novartis). In a number of cases undergoing transplantation at BPRC, prospective recipients

Table 1 Summary of pharmacokinetics[a]

Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (h)	24-h trough (ng/ml)	24-h AUC (ng.h/ml)	C_{max}/dose (ng kg/mg.ml)	Trough/dose (ng kg/mg ml)	AUC/dose (ng h.kg/mg ml)
Cynomolgus monkey: oral administration in microemulsion preconcentrate							
25	800 ± 160	2.0 ± 0.0	69 ± 17	7200 ± 1800	32 ± 6	2.7 ± 0.7	290 ± 70
50	1080 ± 210	2.2 ± 1.1	130 ± 50	10600 ± 3400	22 ± 4	2.5 ± 1.1	210 ± 70
100	1320 ± 230	4.8 ± 1.8	200 ± 40	17800 ± 2600	13 ± 2	2.0 ± 0.4	180 ± 30
150	1720 ± 150	4.2 ± 2.5	450 ± 170	24100 ± 2900	11 ± 1	3.0 ± 1.2	160 ± 20
Rhesus monkey: intramuscular administration in oily solution							
2.5	280 ± 80	2.8 ± 1.5	160 ± 10	4850 ± 130	110 ± 30	63 ± 5	1940 ± 50
5.0	690 ± 200	5.3 ± 3.4	300 ± 170	10200 ± 2100	140 ± 40	61 ± 35	2030 ± 410
10	970 ± 430	5.0 ± 3.4	540 ± 320	16300 ± 7500	100 ± 40	54 ± 32	1630 ± 750

[a]Data presented are mean values ± SD. Mean trough values are derived from the samples harvested at 0 and 24 hs on day 3 of treatment

served also as kidney donors, i.e. unilateral nephrectomy was performed in these animals several weeks before they were subjected to transplantation. In other cases and in all transplants performed at Novartis, the donor animal was subjected to bilateral nephrectomy at the day of transplantation and subsequently killed. The two harvested kidneys were subsequently transplanted into two recipients. The harvested kidneys were perfused with cold (4 °C) University of Wisconsin preservation fluid.

Before and after transplantation, animals received daily treatment with cyclosporine according to protocols presented in the results (Table 2). For oral administration, Neoral drinking solution was used, following the procedure described above. At the day of transplantation, animals received cyclosporine by intravenous route using Sandimmune concentrate for infusion (50 mg/ml).

Animals were regularly monitored after transplantation for body weight, clinical chemistry and hematology. At Novartis, animals were also monitored by telemetry, which included blood pressure measurement. Blood determinations were done twice weekly during the first two-three weeks after transplantation, and thereafter at weekly intervals. Following the same schedule, blood was sampled for cyclosporine determination (24-h trough level). Rejection was concluded from increasing serum creatinine and urea concentrations, using arbitrary levels of 500 μmol/l creatinine or 50 mmol/l urea. All cases with rejection were terminated. Rejection was confirmed by histopathology of the graft taken at autopsy. In the study at BPRC, animals with rejection-free survival were killed around day 100 after transplantation. Rejection-free survival was confirmed by graft histology. In the study at Novartis, animals with > 100 day rejection-free survival were not killed. In some of the animals, the absence of rejection was confirmed by histopathology of a biopsy taken under ultrasound guidance. Untreated controls at BPRC ($n = 4$) rejected their allograft on day 4, 6, 6, and 8 after transplantation. All three controls at Novartis rejected their allografts on day 8 after transplantation.

Determination of cyclosporine

Cyclosporine concentrations in whole blood samples were determined in duplicate with a radio-immunoassay using a monoclonal antibody specific for the parent compound (detection limit 25 ng/ml) [1]. In the pharmacokinetic experiment, the 24-h area-under-the-curve (AUC), C_{max}, and T_{max} was determined (for the AUC following the linear trapoizodal rule). In these experiments, the 24-h trough concentration was calculated as the mean value of the 0-h and 24-h level assessed on the third day of exposure. For C_{max}, trough concentration and AUC, an estimate of the dose-normalized value (normalization to 1 mg/kg dose) was calculated as well.

Results

Pharmacokinetics

The pharmacokinetic profile of cyclosporine concentrations determined on the fourth day after daily administration is presented, for oral administration, in Fig. 1, and for intramuscular administration in Fig. 2. Mean values of pharmacokinetic parameters are presented in Table 1.

Cyclosporine was not detectable in blood samples taken at the start of each course of 4-day administration. During the first 3 days there was a quick building-up of 24-h trough levels, reaching a steady state at the start of the day on which the profile was determined (data not illustrated). For oral administration, maximal concentrations were reached 2 h – 4 h after administration, with a few exceptions, and gradually declined thereafter. Drug levels showed a rather large variation between cases in individual groups. This variation appeared not to be associated with intrinsic individual characteristics; the same animal could show relatively high levels with the first dose, and relatively low levels in the subsequent investigation with the second dose (e.g., animals C1 and C9). The opposite was also documented, e.g. animal C6 showed relatively low concentrations after exposure to the first dose (100 mg/kg), and relatively high levels in the subsequent investigation with the second dose (150 mg/kg). The levels of C_{max}, trough concentration and 24-h AUC were dose-dependent. However, the dose-normalized values revealed for C_{max} an about 3-fold decrease with increasing dose between the 25 mg/kg and 150 mg/kg dose. A somewhat lower decrease with increasing dose was observed for 24-h AUC. The trough concentration showed an acceptable linear relationship with the dose. Combining all doses, the mean dose-normalized value ± SD was for C_{max} 20 ± 9 ng kg/mg ml, for the trough concentration 2.6 ± 0.9 ng kg/mg ml, and for the 24-h AUC 210 ± 70 ng h kg/mg ml. Finally, the T_{max} was about twofold higher in the groups treated

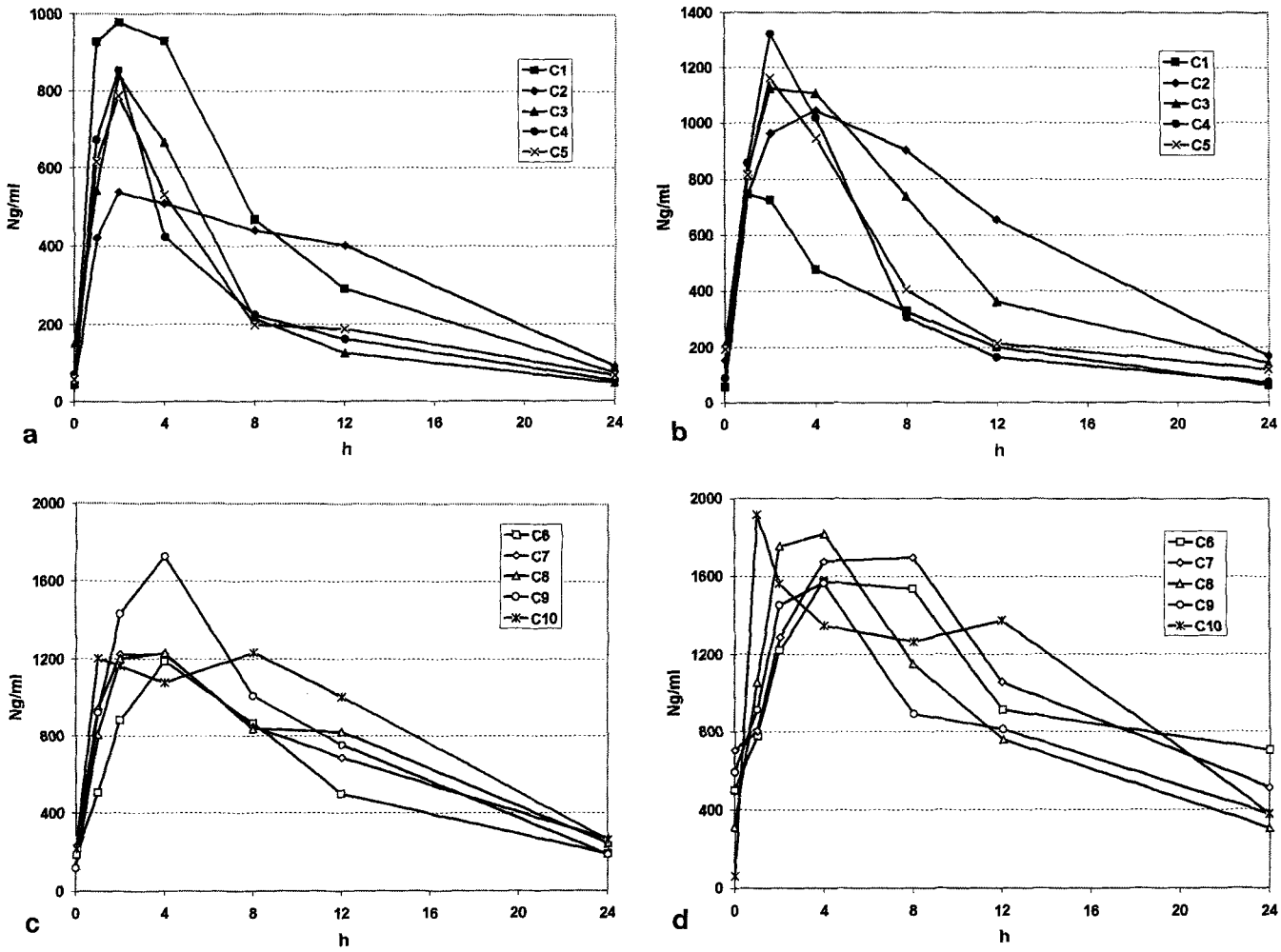

Fig. 1 Pharmacokinetic profile of cyclosporine upon oral administration as microemulsion preconcentrate to cynomolgus monkeys, assessed on day 4 of daily administration, at doses of 25 mg/kg **a**, 50 mg/kg **b**, 100 mg/kg **c** and 150 mg/kg **d**

with 100 mg/kg and 150 mg/kg than in the groups treated at 25 mg/kg and 50 mg/kg.

The profiles after intramuscular administration (Fig. 2) differed largely from those after oral administration. There was a more gradual rise to maximum levels, and a clear peak was not evident. This difference from oral administration is most likely due to depot formation after intramuscular administration in oil, causing a more gradual release of the drug into the circulation. The T_{max} values were higher for intramuscular administration than for oral administration. Regarding intramuscular administration, there was furthermore a variation between individual animals in individual groups, especially concerning C_{max} values. Male animals (R1, R4, R5 and R8) and female animals (R2, R3, R6 and R7) did not show a clear difference in this respect. Also the 24-h AUC levels varied, in particular in the groups dosed at 10 mg/kg per day. This variation proved, on the one hand, not to be related to intrinsic characteristics of specific animals, e.g. case R2 showed relatively high concentrations after exposure to the first dose (10 mg/kg), and relatively low levels in the subsequent investigation with the second dose (2.5 mg/kg). On the other hand, there was one animal (R8) revealing much higher concentrations than other animals in the same group, both after the first exposure to 5 mg/kg and after the second exposure to 10 mg/kg. It is not clear whether this is an incidental event in the study, or if it represents an intrinsically higher absorption characteristic of this animal. Similar to oral administration, the 24-h AUC, C_{max} and the 24-h trough concentration were dose-dependent. The dose-dependency of C_{max} and the 24-h AUC was more linear than that observed for oral administration. Combining all doses, the mean dose-normalized value ± SD was for C_{max} 110 ± 40 ng kg/mg ml, for the 24-h trough concentration 58 ± 27 ng kg/mg ml, and for the 24-h AUC 1810 ± 580 ng h kg/mg ml. These values are about 5.5 fold (C_{max}), 22 fold (24-h AUC)

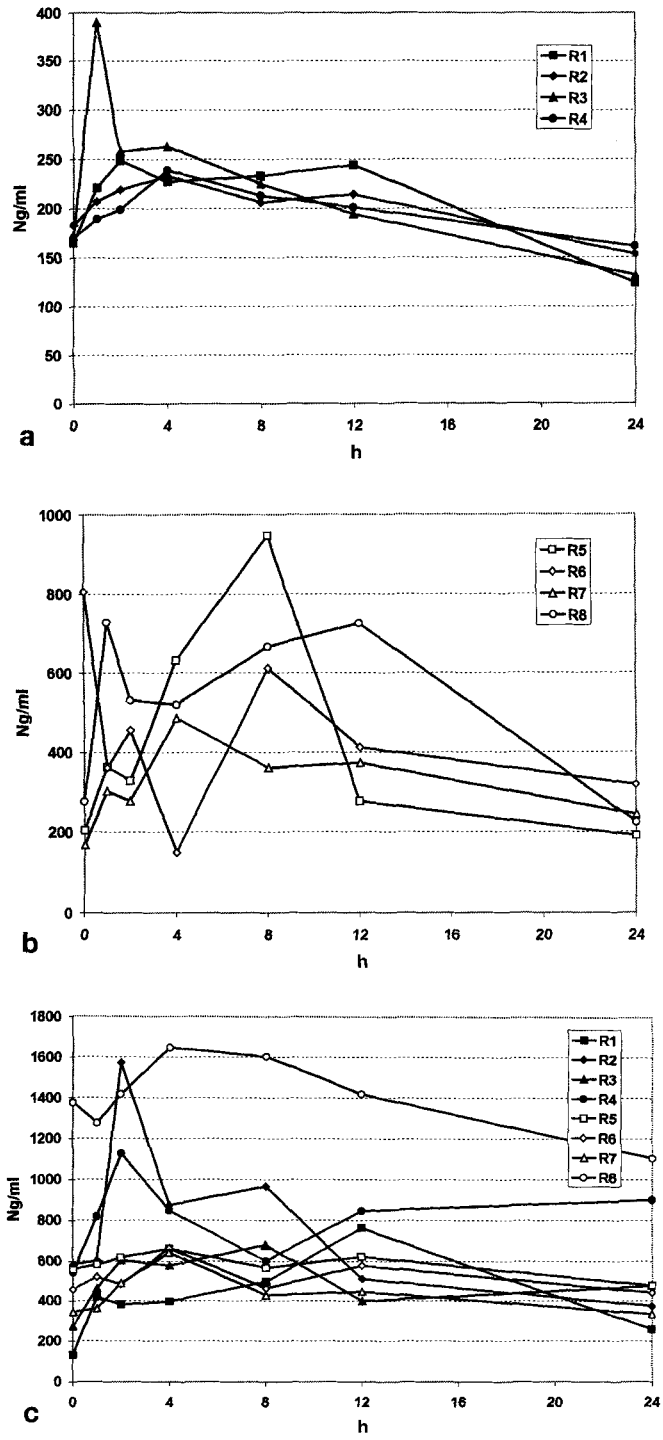

Fig. 2 Pharmacokinetic profile of cyclosporine upon intramuscular administration in oily formulation to rhesus monkeys, assessed on day 4 of daily administration, at doses of 2.5 mg/kg **a**, 5.0 mg/kg **b**, and 10.0 mg/kg **c**

and 9 fold (24-h trough level) higher than for oral administration, respectively. Finally, the T_{max} was somewhat higher in the groups treated at 5 mg/kg and 10 mg/kg than in the group treated at 2.5 mg/kg.

Transplantation experiments

Data on allograft survival and cyclosporine trough concentrations in animals in different treatment groups are presented in Table 2. Based on the data of the pharmacokinetic experiment, the standard protocol for optimal cyclosporine immunosuppression was designed as a daily oral dose of 150 mg/kg given at the day before transplantation and subsequently during the first two weeks after transplantation, an intravenous dose of 20 mg/kg body weight given at the day of transplantation, and a reduction in daily oral dose to 100 mg/kg body weight starting two weeks after transplantation: notably, no steroids were given in any of the animals. Using this protocol, 8 out of 11 animals (BPRC, 4 out of 5 animals; Novartis, 4 out of 6 animals) showed > 100 day survival without biochemical or histological signs of rejection.Three animals rejected their allograft at about 4 weeks after transplantation. As reported elsewhere [24], two cases with rejection-free survival showed a malignant lymphoma at autopsy, indicative of potential overimmunosuppression. Three additional cases had to be subjected to protocol amendments: in two cases, the cyclosporine dose had to be reduced to 75 mg/kg because of an increase in blood pressure, and the third case did not tolerate daily oral drug administration and was changed to daily intramuscular administration at 20 mg/kg cyclosporine. Cyclosporine 24-h trough concentrations showed a wide variation within and between individual animals: average levels in individual animals varied between 260 and 650 ng/ml during the 150 mg/kg dosing phase, and between 210 and 700 ng/ml during 100 mg/kg maintenance. Trough levels did not differ between animals with rejection and animals showing > 100 day rejection-free survival. For instance, one case with rejection on day 32 after transplantation showed quite high cyclosporine concentrations (mean ± SD during the 150 mg/kg treatment period 650 ± 257 ng/ml, during the subsequent maintenance phase up to 1200 ng/ml), whereas cases with rejection-free survival could show average values around 250 ng/ml. Finally animals with presumed cyclosporine-associated side effects (lymphoma development, blood pressure increase) showed concentrations in the same range as animals without these side effects, e.g. in two animals that developed a lymphoma, the average trough level during the 100 mg/kg treatment period was 429 ± 169 ng/ml and 700 ± 140 ng/ml, respectively. The only effect noted was the change in one animal from oral dosing to intramuscular administration: this animal

Table 2 Kidney allograft survival in cynomolgus monkeys and trough concentrations of cyclosporine

Treatment	Number of animals (day)		Cyclosporine concentration in individual animals, related to dose level, mean ± SD	
	Rejection	Survival > 100 days		
150 mg/kg *po* day –1 and day 1–14, 20 mg/kg *iv* day 0, 100 mg/kg *po* since day 15			150 mg/kg	100 mg/kg
		8	259 ± 163	246 ± 203
			364 ± 271	240 ± 119
			452 ± 121	413 ± 182
			489 ± 224	429 ± 169
			535 ± 187	298 ± 167
			571 ± 307	548 ± 308
			573 ± 582	290 ± 141
			614 ± 280	700 ± 140
	3		384 ± 216	208 ± 83
	(Day 26, 26, 32)		493 ± 133	641 ± 252
			650 ± 157	1207 ± 243
Same, but reduction to 75 mg/kg during day 8–56, or reduction to 75 mg/kg starting day 33 ($n = 2$)			150 mg/kg	75 mg/kg
		2	509 ± 211	431 ± 230
			635 ± 307	512 ± 230
Same, but change to 20 mg/kg *im* starting day 23			150 mg/kg	20 mg/kg *im*
		1	290 ± 299	1015 ± 175
100 mg/kg *po* day –1 and day 1–14, 10 mg/kg *iv* day 0, 30 mg/kg *po* since day 15			100 mg/kg	30 mg/kg
		2	239 ± 133	114 ± 48
			332 ± 96	124 ± 96
	2		159 ± 95	229 ± 161
	(Day 24, 28)		398 ± 54	131 ± 40
30 mg/kg *po* day –1 and day + 1 onwards, 10 mg/kg *iv* day 0			30 mg/kg	
	5		43 ± 8	
	(Day 9, 10, 22, 70, 91)		49 ± 17	
			67 ± 108	
			139 ± 81	
			221 ± 165	
20 mg/kg *po* day –1 and day + 1 onwards, 4 mg/kg *iv* day 0, reduction to 10 mg/kg starting day 15 ($n = 1$) or day 41 ($n = 1$)			20 mg/kg	10 mg/kg
	3		26 ± 13	21 ± 1
	(Day 10, 24, 69)		29 ± 6	27 ± 15
			30 ± 12	
10 mg/kg *po* day –1 and day + 1 onwards, 4 mg/kg *iv* day 0			10 mg/kg	
	6		15 ± 1	
	(Day 8, 8, 10, 15, 22, 26)		18 ± 10	
			19 ± 7	
			20 ± 18	
			38 ± 23	
			41 ± 60	

showed persistently higher cyclosporine concentrations during the intramuscular treatment period (1015 ± 175 ng/ml), without apparent side effects. Notably, the standard deviation in the values determined during the intramuscular administration period was much lower than that of levels determined during oral administration, indicating a more consistent exposure.

One group of four animals was studied using a lower dose, i.e. a daily oral dose of 100 mg/kg given at the day before transplantation and subsequently during the first two weeks after transplantation, an intravenous dose of 10 mg/kg body weight given at the day of transplantation, and a reduction in daily dose to 30 mg/kg body weight starting two weeks after transplantation. Following this protocol, two animals rejected their graft during the fourth week after transplantation, and two cases showed > 100 days rejection-free survival without biochemical or histological signs of rejection. Mean cyclosporine trough concentrations ranged between 160 and 400 ng/ml during the first two weeks of treatment (100 mg/kg), and, in the two animals with long-term rejection-free survival, they were about 120 ng/ml during the 30 mg/kg treatment period thereafter. These values fit to the data of the pharmacokinetic experiment. The average cyclosporine trough levels in animals which rejected their graft were in the same range or even somewhat higher.

A third group of animals was treated with a dose of cyclosporine that was presumed to be suboptimal, based on the results of the pharmacokinetic experiment, i.e. a dose ranging between 10 and 30 mg/kg body weight given at one day before transplantation and from the day after transplantation on, with an intravenous dose of 4–10 mg/kg at the day of transplantation. None of these animals revealed long-term rejection-free survival, but the survival times were longer than those of untreated controls, especially of animals treated at an intial dose of 20–30 mg/kg. Rejection-free survival of more than two months was noted in three animals, two being on 30 mg/kg maintenance treatment (70 and 91 days, respectively) and one being on 10 mg/kg maintenance treatment (69 days). Mean cyclosporine 24-h trough concentrations in the long-term surviving animals on 30 mg/kg were 139 and 221 ng/ml, respectively, which is higher than those of three animals that rejected their grafts earlier after transplantation (average levels ranging between 43 and 67 ng/ml). Remarkably, the animal on 10 mg/kg maintenance that rejected its allograft on day 69 had an average cyclosporine level of 27 ng/ml, which was in the same range as other cases with earlier rejection. Animals that received 10 mg/kg cyclosporine as maintenance starting on the day after transplantation all rejected their allograft within the first months after transplantation, and showed average cyclosporine trough concentrations between 15 and 41 ng/ml.

Discussion

Chronic intramuscular administration of cyclosporine in oily formulation was commonly used in studies on non-human primates, but is nowadays preferably avoided because of potential side effects, such as tissue reactions at the injection site. Therefore, oral administration is gaining in preference. This prompted us to compare the pharmacokinetics of cyclosporine in oil given intramuscularly, with those of the drug delivered orally as microemulsion formulation, and to evaluate the efficacy of cyclosporine given orally as monotherapy in kidney transplantation experiments. For logistical reasons, cynomolgus monkeys were used in studies on oral administration (pharmacokinetics and transplantation experiments), and rhesus monkeys were used in the pharmacokinetic study using intramuscular administration. Since we are not aware of differences between these two species in pharmacokinetics and efficacy of immunosuppressants like cyclosporine, it seems possible to compare the effects of oral and intramuscular administration in these different species. To our knowledge, transplantation experiments using cyclosporine monotherapy have not been performed on rhesus monkeys, and we therefore only present data of such studies on cynomolgus monkeys.

The pharmacokinetic experiment showed that intramuscular administration is associated with a higher absorbtion than oral administration. Normalized to a 1 mg/kg dose, the 24-h AUC is about 9 times higher, the 24-h trough concentration about 22 times higher, and the C_{max} about 5.5 times higher compared to oral administration. The slow release from an intramuscular depot might underly that this higher exposure is most pronounced for the trough concentration, and least pronounced for C_{max} values. We conclude that at similar 24-h trough concentrations, exposure expressed by 24-h AUC is about twice as high for intramuscular administration in oil than for oral administration in microemulsion formulation. Both oral and intramuscular administration are associated with a rather high inter-individual variability (e.g., variation coefficient of 24-h AUC about 20–40 % in distinct groups): this is not directly expected, as lower absorption following oral administration might be associated with a higher variability in exposure, compared to intramuscular administration.

In extrapolation of these data from monkeys to humans, it is evident that much higher doses of orally administered cyclosporine in microemulsion preconcentrate are needed in monkeys to obtain blood concentrations in the therapeutic range. This phenomenon fits in with literature reports mentioned above on cyclosporine administered orally in oily solution [26, 29]. Apparently, the oral absorption characteristics of cyclosporine in monkeys are similar for the oily formulation and for the microemulsion preconcentrate.

Transplantation experiments enable to relate the exposure data with efficacy in immunosuppression. For intramuscular administration of cyclosporine to rhesus monkeys it has been reported previously that a daily doses of 10 mg/kg and 25 mg/kg for three weeks combined with pretransplant blood transfusions and/or a

three-week course of azathioprine (2 mg/kg daily) and prednisolone (1 mg/kg daily) resulted in graft survival: upon discontinuation of immunosuppression, all animals subsequently rejected their graft (median survival in different groups varying between 32 and 55 days) [2]. Upon prolonged cyclosporine administration for 6–12 months, a condition is achieved in which grafts can survive without the necessity of maintenance immunosuppression [11, 13]. Thus, a dose of 10 mg/kg or more given intramuscularly in these double/triple treatment protocols appears to be in the immunosuppressive range. Based on the pharmacokinetic data, the 24-h trough concentration is expected to be around 500 ng/ml or more. This level thus appears to be in the immunosuppressive range: lower 24-h trough concentrations ranging between 40 and 200 ng/ml after intramuscular administration to cynomolgus monkeys is invariably associated with early kidney allograft rejection [28].

Using oral administration of cyclosporine to cynomolgus monkeys as monotherapy, a two-week daily treatment at 150 mg/kg followed by dose reduction to 100 mg/kg resulted in long-term rejection-free survival in 8 of 11 animals studied (Table 2). Three other cases were subjected to dose reduction (75 mg/kg per day) or change to intramuscular administration, and also showed > 100 days rejection-free survival. At these doses, the 24-h trough concentrations ranged between 200 ng/ml and 700 ng/ml. The trough concentrations apparently were not related to the occurrence of rejection or rejection-free survival in this treatment group, and not to the occurrence of side effects that can be ascribed to cyclosporine immunosuppression, e.g. lymphoma development (two cases) or increase in blood pressure (two other cases). As an illustration, the single case that was converted to daily 20 mg/kg intramuscular administration can be mentioned, which showed a mean cyclosporine concentration of 1015 ng/ml and did not manifest side effects. In contrast to the data from the pharmacokinetic experiment, the variability in levels appeared to be lower in this animal during intramuscular cyclosporine dosing than that in animals subjected to oral administration. Rejection-free survival was also noted in 2 of 4 animals treated at a lower dose, i.e. 100 mg/kg during the first two weeks after transplantation followed by dose reduction to 30 mg/kg: 24-h trough concentrations in these animals ranged between 50 ng/ml and 430 ng/ml during the first two weeks and between 50 ng/ml and 170 ng/ml thereafter. Also, in this group there was no unequivocal correlation between cyclosporine 24-h trough concentration and rejection-free survival.

At lower doses, presumed to be suboptimal, i.e. daily oral doses of 30 mg/kg, all 5 cases studied showed rejection, but with quite variable rejection-free survival rates ranging from 9 to 91 days. In this group, two cases with prolonged rejection-free allograft survival showed a mean 24-h trough level of 139 and 221 ng/ml, respectively, whereas average levels below 100 ng/ml were associated with shorter survival times. Cyclosporine at even lower dose levels, i.e. 10–20 mg/kg associated with 24-h mean blood concentrations between 15 ng/ml and 40 ng/ml, resulted in graft rejection within 4 weeks after transplantation. A remarkable exception was one single case which first rejected on day 69 after transplantation and had average trough concentrations of 27 ng/ml. From these data, we conclude that average 24-h trough levels above 100 ng/ml are minimally required to yield sufficient immunosuppression associated with prolongation of graft survival.

In conclusion, cyclosporine blood levels in an apparent therapeutic range can be obtained in monkeys by oral administration of the compound in microemulsion preconcentrate, and long-term rejection-free survival can be achieved using oral cyclosporine monotherapy, notably even in the absence of steroids. Compared to other species including man, much higher dosages of the compound given orally have to be administered to reach therapeutic levels. The pharmacokinetic profiles for intramuscular and oral administration differ widely, which has consequences for interpretation of 24-h trough levels with respect to immunosuppressive dose. The present data indicate that higher trough levels are necessary for intramuscular administration than for oral administration to reach a similar therapeutic effect, and that upon oral administration, trough levels above 100 ng/ml are minimally required to achieve relevant, rejection-free survival of a kidney allograft.

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