TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Immunosuppressive properties of a series of novel inhibitors of the monocarboxylate transporter MCT-1

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Keywords
alloimmune responses, immunosuppression, monocarboxylate transporter, operational tolerance.

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Conflicts of Interest
Clare M. Murray, Douglas Ferguson, Robert V. Bundick and David K. Donald were employed at AstraZeneca R&D Charnwood, Loughborough, Leicester, UK. No other potential conflict of interest for any author.

Received: 13 March 2012
Revision requested: 21 May 2012
Accepted: 14 September 2012
Published online: 8 November 2012

doi:10.1111/j.1432-2277.2012.01579.x

Summary

We have recently described the immunosuppressive properties of AR-C117977 and AR-C122982, representatives of a group of compounds identified as inhibitors of lactate transporters (monocarboxylate transporters; MCTs). These compounds demonstrate the potential therapeutic usefulness of inhibiting MCT-1, but their physical and metabolic properties made them unsuitable for further development. We have therefore tried to find analogues with similar immunosuppressive efficacy and a more suitable profile for oral administration. Five analogues of AR-C117977 were synthesised and screened for binding to the transporter, for inhibition of proliferation of both human and rat lymphocytes, for *in vivo* activity in a model of graft-versus-host (GvH) response in the rat, and in high- and low-responder cardiac transplant models in the rat. There was a good correlation between levels of binding of the five analogues to MCT and their inhibition of lymphocyte proliferation in human and rat cells. Furthermore, activity in both the GvH response and the cardiac transplant models correlated well with the determined concentrations of test compound in plasma. These findings on new analogues of MCT-1 inhibitors have taken us further towards defining the pharmacokinetic properties that may help to identify future drug candidates among inhibitors of MCT-1.

Introduction

In recent decades, improvements in the efficacy of immunosuppressive drugs, with successful prevention of acute rejection (AR), have reduced the interest in AR and shifted interest towards issues of long-term graft survival and chronic dysfunction. Calcineurin inhibitors (CNIs), the basis of most immunosuppressive regimens used today, are associated with nephrotoxicity. The extent of the influence of chronic CNI-related nephrotoxicity on the long-term success rate is under current debate [1,2]. Currently, reduced dosing of CNI (preferably tacrolimus), made possible by inclusion of mycophenolate mofetil and induction therapy, is the immunosuppression regimen most commonly used [3,4]. Even so, the major goal in the field of transplantation and immunosuppression continues to be to discover drugs with equivalent immunosuppressive efficacy, but without the toxicities that reduce long-term graft function and survival.

In the search for low molecular-weight compounds with immunosuppressive activity that lack the adverse effects of current clinically used drugs, we discovered a series of compounds with potent antiproliferative activity and with a different profile of action to those of the currently used

immunosuppressive drugs [5]. Attempts to determine the nature of the receptor and the mechanism of action of these compounds implicated the monocarboxylate transporter (MCT-1), which is known to be important for the transport of lactate, pyruvate and ketone bodies across cell membranes. The association of MCT-1 with lymphocyte proliferation remained completely unknown, and prompted other studies to determine the importance of this discovery. Two compounds that were found to bind to MCT-1, AR-C177977 and AR-C122982, were efficacious in alloimmune responses, such as the graft-versus-host (GvH) response, and in preventing cardiac allograft rejection in rats and mice [6,7] as well as in nonvascularized transplant models [8]. Animals treated with these compounds had no overt adverse reactions; however, atrophy of the testis was observed on termination of the experiments. A more recent investigation [9] has confirmed our initial observation showing the usefulness of MCT-1 inhibition in preventing allograft rejection. It has improved our understanding of the mechanism of action of MCT-1 inhibitors in creating a tolerogenic environment for the allograft by preferentially targeting T-effector cells while sparing the generation of T-regulatory cells.

There are four closely associated MCTs (designated MCT 1–4) that have variable expression in different tissues, for example, in muscle, testis and retina. The compounds in this and our previous study were examined for inhibition of the four isoforms of MCT; activity was found to be confined to MCT-1 and to a lesser extent to MCT-2. With a view to obtain orally active compounds, five analogues of AR-C117977 were chosen based on their binding activity, their *in vitro* efficacy in inhibiting lymphocyte proliferation, and their pharmaco-kinetic (PK) properties, for evaluation of their immunosuppressive activity *in vivo* (Table S1). The compounds were tested in a GvH response model and in low- and high-responder cardiac transplant models in the rat.

Materials and methods

Compounds

AR-C141990, AR-C148990, AR-C155187, AR-C152593 and AR-C155858 were synthesised in the Medicinal Chemistry laboratories of AstraZeneca R&D Charnwood. For binding and *in vitro* proliferation assays, the compounds were dissolved in dimethyl sulphoxide at a concentration of 10 mM and then diluted in assay buffer or culture medium.

Lipophilicity (logD) was measured as the distribution coefficient between 1-octanol and aqueous buffer, $\log D_{O/W}$, at pH 7.4; compounds were quantified using HPLC with quantitative mass spectrometry (MS) as a method to measure the relative octanol and aqueous concentrations. Solubility was measured at 20°C in sodium phosphate buffer pH 7.4 by generation of a saturated solution of the compound, followed by assaying the solution using HPLC with UV quantification and MS identification.

Radio-ligand binding assays for MCT-1 and MCT-2 expressed in yeast membranes, lactate transport in Ins1 cells expressing MCT-3 and MCT-4 and rat and human lymphocyte proliferation assays, were performed according to methods described previously [5]. The method for radioligand binding to Jurkat T-cell membranes using a scintillation proximity assay has also been described previously [10].

For the heart allograft models

Suspensions of the drugs were prepared in 5% Tween 20 (v/v) and carboxymethyl cellulose solution and stored at –20°C. Four different concentrations were prepared: 3, 10, 30 and 100 mg/ml. The suspensions were mixed thoroughly before administration. Subcutaneous injections were performed once a day from day 0 to day 9, and 0.2 ml of a specific suspension was injected into rats weighing 200 g to reach the target dose. In addition, AR-C155858 was administered orally.

Cyclosporine A (CsA; Sandimmun® 100 mg/ml; Novartis, Basle, Switzerland) was dissolved in Intralipid® (200 mg/ml; Pharmacia & Upjohn, Lund, Sweden) to a final concentration of 4 mg/ml and given orally for either 10 or 40 days [6]. Doses of 10 mg/kg p.o. in a volume of 0.5 ml were given once a day to rats weighing 200 g.

Animals

In vitro proliferation studies and GvH studies

Male DA, DA/Lewis and Lewis rats were obtained from Bantin and Kingman (Hull, UK). The rats were conditioned for at least 1 week in purpose-built housing before use. They were housed in standard cages with water and chow available *ad libitum*, with controlled cycles of light and darkness. All experiments were performed in accordance with UK Home Office regulations.

Transplantation studies

Isogenic rats were obtained from Mollegaard Breeding and Research Centre (Ejby, Denmark) and conditioned for at least 1 week before transplantation at our laboratory. They were housed in standard cages under controlled light/dark cycles and fed a standard laboratory diet with free access to water. Male rats of the following strains were used: DA ($RT1^{avl}$), PVG ($RT1^{c}$), and WF ($RT1^{u}$). Recipients weighed 180–240 g and donors weighed 100–160 g. The study design was approved by the Research Ethics Committee of Lund University, and all procedures were performed in accordance with the Good Laboratory Practice code published by the National Board of Health and Welfare in Sweden.

Graft-versus-host response

The protocol was based on a method described previously [11]. Briefly, single cell suspensions were prepared from spleens of DA and DA/Lewis rats. DA/Lewis recipient rats received approximately 1×10^7 DA cells into the right hind paw and approximately 1×10^7 DA/Lewis cells into the left. Compounds or vehicle were dosed daily by the oral or subcutaneous route (six animals per group). On day 7, the weight of the right popliteal lymph node receiving stimulator cells was compared with the weight of the left popliteal lymph node receiving isogeneic cells.

Heart transplantation

The donor and recipient operations were performed under clean, but not sterile conditions. The rats were anaesthetized with intraperitoneal injection of chloral hydrate or Dormicum®/Hypnorm®. The donor heart was flushed with cold Perfadex® (Pharmacia & Upjohn), and the caval and pulmonary veins were ligated before removal. The heart was heterotopically transplanted to the right neck vessels of the recipient, the aortic root being anastomosed to the common carotid artery and the pulmonary artery to the jugular vein with nonsuture cuff technique [12]. In retransplants, the second graft was placed in the groin and anastomosed to the femoral vessels. Allograft function was monitored twice a day by palpation, with rejection being defined as cessation of a palpable heart beat.

Toxicity

As a crude evaluation of signs of toxicity, the postoperative development of body weight was used.

Statistical analysis

Graft survival was plotted according to Kaplan–Meier and differences between groups were compared using log-rank statistics. In the GvH response model, differences between dosed rats and control rats were compared using one-way analysis of variance followed by the Dunnett multiple comparison test.

Results

Compounds: physical and pharmaco-kinetic properties

The properties of the compounds are given in Table 1a, with the two earlier-described compounds (AR-C177977 and AR-C122982) included for comparison. The early compounds were very lipophilic and, associated with this, their solubilities in water were low and protein binding was high. Both early compounds also exhibited high clearance in rat PK studies, with short plasma half-lives and very low oral bioavailability – consistent with their physicochemical properties (Table 1a).

Our aim was to reduce the lipophilicity of the molecules and thereby increase solubility and improve the PK properties. As can be seen from the tabulated results, it was possible to dramatically improve solubility and to increase the half-lives of the compounds; however, clearance still remained higher than desired and bioavailability remained low. Even so, these analogues were an important step in improvement of the properties of the compounds towards the desired profile.

Binding and functional assays

The binding activity and functional activity of the new compounds relative to those of the two earlier compounds are shown in Table 1b. There was a reduction in binding affinity in comparison to the earlier compounds, but there was an excellent correlation between binding activity and functional activity. As reported earlier for AR-C117977 and AR-C122982, the compounds also bound to MCT-2 expressed in yeast, but with approximately 10 times less potency. No functional inhibition of lactate transport in cells expressing MCT-3 or 4 was observed (Table 1b).

Table 1a. Physical and pharmaco-kinetic properties of the MCT-1 inhibitors tested.

		Solubility	Hu PPB	Rat PPB	Rat plasma PK (i.v.)	Rat plasma PK (i.v.)	Rat plasma PK (p.o.)
AR-C	Log D	(μM)	(% Free)	(% Free)	Cl (ml/min*kg)	Half-life (h)	Oral bioavailability (%)
117977	4.8	0.17	0.1	0.05	>100	1.4	<3
122982	4.8	<0.002	0.3	0.5	97	0.4	<3
141990	1.6	972	36	40	67	0.7	3
148990	2.4	ND	9	19	50	0.4	3
155187	2.2	47.2	25	46	50	2.7	17
152593	2.3	518	37	52	48	1	8.5
155858	1.1	6305	49	34	38	9.6	4

Log D, lipophilicity; Hu PPB, human plasma protein binding; Cl, clearance; i.v., intravenous; p.o., peroral.

Table 1b. *In vitro* efficacy and selectivity in ligand binding and functional assays.

AR-C	Jurkat binding pKi	Human PMA pIC50	Rat PMA pIC50	MCT-1 binding pKi	MCT-2 binding pKi	MCT-3 transport pIC50	MCT-4 transport pIC50
117977	9.5 ± 0.4	9.6 ± 0.4	9.6 ± 0.2	9.3 ± 0.6	7.8 ± 0.6	<5	<5
122982	10.5 ± 0	9.5 ± 0.2	9.0 ± 0.2	9.8 ± 0.4	8.3 ± 0.5	<5	<5
141990	8.3 ± 0.3	8.5 ± 0.5	7.9 ± 0.2	7.6 ± 0.1	6.6 ± 0.3	<5	<5
148990	9.1 ± 0.3	9.0 ± 0.3	8.6 ± 0.2	8.3 ± 0.5	7.9 ± 0.6	<5	<5
155187	9.3 ± 0.4	9.8 ± 0.4	9.0 ± 0.3	8.3 ± 0.4	6.6 ± 0.1	<5	<5
152593	8.5 ± 0.2	8.8 ± 0.3	8.0 ± 0.2	7.6 ± 0.2	6.6 ± 0.2	<5	NT
155858	8.9 ± 0.3	9.2 ± 0.1	8.6 ± 0.2	8.3 ± 0	7 ± 0.2	<5	<5

Data are mean ± SD from ≥3 independent experiments or mean ± range from two experiments. PMA, phorbal myristate acetate and ionomycin-stimulated lymphocyte proliferation.

Graft-versus-host response

All the compounds gave a dose-related inhibition of the response in the GvH model in the rat. Figure 1 shows a comparison with earlier compounds; the ratio of the plasma concentration of a compound to its binding affinity is plotted against the degree (percentage) of inhibition. Inhibition of the response was achieved in all cases where the plasma concentration was in excess of the K_d for binding. This is consistent with our earlier results for AR-C117977 and AR-C122982 (Fig. 1).

Long-term cardiac graft survival in high-responder recipients

Untreated PVG rats that received DA cardiac transplants had a median graft survival time of 7 days (range 6–8) (Table 2a). Oral CsA given for 10 days resulted in a median graft survival of 20.5 days. Acute rejection was prevented long-term (>100 days) in this high-responder rat combination when treated with AR-C148990 (30 mg/kg s.c.), AR-C155187 (30 or 100 mg/kg s.c.), or AR-C117977 (30 mg/kg s.c.). In contrast, treatment with AR-C141990 (100 mg/kg s.c.), AR-C155 858 (100 mg/kg s.c.), or AR-C 152593 (30 mg/kg s.c.) had a moderate prolongation of graft survival similar to that of CsA given for 40 days. As the PK analyses of 10 days of subcutaneous injections of the MCT-1 inhibitor (AR-C117977) had previously shown the presence of compound in plasma for up to 40 days [6], we included a group of animals that received CsA for 40 days after transplantation, resulting in a median time of graft survival of 52 days. Compared to this CsA group, AR-C155187 in doses of 30–100 mg/kg was statistically significantly superior.

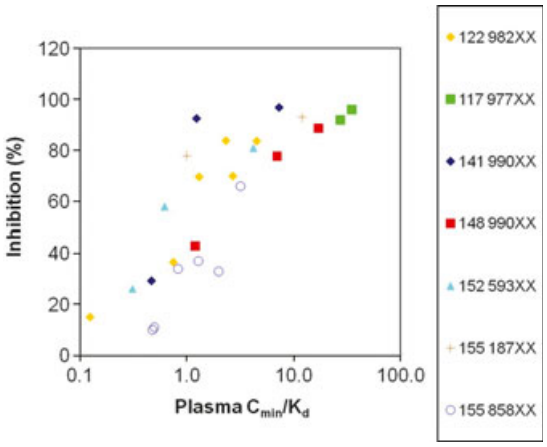

Figure 1 Ratio of the plasma concentration of a compound to its binding affinity (P/K_d) plotted against percentage inhibition. There is also a comparison with earlier compounds (AR-C122982 and AR-C117977).

Long-term cardiac graft survival in low-responder recipients

Reversing the donor/recipient strains creates a low-responder combination with minimal requirement of immunosuppression to prevent AR [13]. Median graft survival in untreated DA rats was 8 days (range 7–8) (Table 2a). Given CsA at 5 mg/kg/day, which was half of the normal dose, all animals had long-term functioning of grafts (>100 days). Likewise, with low doses of AR-C155858 (30 mg/kg s.c.), AR-C141990 (30 mg/kg/day), or AR-C148990 (30 mg/kg/day), all grafts were protected from rejection in the long term. The only exceptions were two animals that had been treated with AR-C148990 and AR-C155858, respectively, and had rejection at 14 and 42 days.

Long-term graft survival after re-transplantation

A second heart from the same donor strain was transplanted to animals that had primary graft survival of >100 days. All secondary grafts survived >50 days after the time of retransplantation without any further immunosuppression, thus showing evidence of donor-specific tolerance (Table 2b). This was seen in both high- and low-responder combinations.

Association between plasma concentration and graft survival

Table 3 gives the minimum receptor occupancy calculated from the plasma or blood levels of the compound during the dosing period (day 3–day 6) and after dosing had ceased (day 12). From this, it is clear that in studies when minimum occupancy is low [e.g. for AR-C141990 dosed at 3 mg/kg (occupancy 20%) or AR-C152593 dosed at 10 mg/kg (occupancy 6%)], rejection occurs within the same time scale as in untreated control animals. There is no evidence of immunosuppressive efficacy at these low doses, a result that is fully in accordance with the GvH results. In contrast, where occupancy was high both during the dosing period and at day 12, long-term graft survival was achieved (illustrated by AR-C155187 at 30 mg/kg, with 96% occupancy). Where occupancy was high during the dosing period, but where the levels of compound declined rapidly on cessation of dosing, the graft was maintained during the dosing period, but long-term graft survival was not achieved (as illustrated by AR-C155858 given orally at 100 mg/kg).

Table 2a. Graft survival of rat cardiac transplantation.

Groups (treatment)	Graft survival (days)	Median (days)	*P*-value (groups compared)
DA to PVG high-responder			
1. No treatment	6 6.5 7 7 8 8	7	
CsA			
2. 10 mg/kg p.o. days 0–9	17 19 19 22 22 24	20.5	
3. 10 mg/kg p.o. days 0–40	10 51 52 53 54 60	52	
AR-C141990 (s.c. inj. days 0–9)			
4. 3 mg/kg	7 7 7 8 8 9	7.5	
5. 10 mg/kg	7 9 9 9 9 23	9	
6. 30 mg/kg	7 9 11 12 15 20	11.5	
7. 100 mg/kg	16 20 26 33 55 >100	29.5	0.05 (7 vs. 2) 0.85 (7 vs. 3)
AR-C148990 (s.c. inj. days 0–9)			
8. 30 mg/kg	11 34 >100 >100 >100 >100	>100	0.01 (8 vs. 2)
AR-C155187 (s.c. inj. days 0–9)			
10. 30 mg/kg	90 >100 >100	>100	0.01 (10 vs. 2) 0.01 (10 vs. 3)
11. 100 mg/kg	55 >100 >100 >100 >100 >100 >100	>100	0.0002 (11 vs. 2) 0.0006 (11 vs. 3)
AR-C117977 (s.c. inj. days 0–9)			
12. 30 mg/kg	>100 >100 >100 >100 >100 >100	>100	
AR-C152593 (s.c. inj. days 0–9)			
13. 10 mg/kg	6 7 7	7	
14. 30 mg/kg	13 15 23 40 48 >150	31.5	
AR-C155858 (s.c. inj. days 0–9)			
15. 100 mg/kg	9.5 12 44 44 46 60	44	
16. 30 mg/kg	7 8 8	8	
17. 10 mg/kg	7		
18. 3 mg/kg	7 7 8	7	
AR-C155858 (p.o. days 0–9)			
19. 200 mg/kg (bid)	3* 6* 8		
20. 100 mg/kg	9 10 10 10 10 68	10	
21. 30 mg/kg	6 7 8 8 9 11	8	
22. 10 mg/kg	6 7 7 7 8 8	7	
PVG to DA low-responder			
23. No treatment	7 7.5 8 8 8 8	8	
CsA (p.o. days 0–9)			
24. 5 mg/kg	>100 >100 >100 >100 >100 >100	>100	
AR-C155858 (s.c. days 0–9)			
25. 30 mg/kg	42 >100 >100 >100 >100		
AR-C141990 (s.c. days 0–9)			
26. 30 mg/kg	>100 >100 >100 >100 >100 >100	>100	
AR-C148990 (s.c days 0–9)			
27. 30 mg/kg	14 >100 >100 >100 >100 >100	>100	

p.o., peroral administration; s.c., subcutaneous administration.
*Death with functioning graft.

Table 2b. Graft survival in tolerant recipients with >100 days of graft survival that received a second heart from either the same donor rat strain or from a third-party donor rat strain. No further treatment.

Groups (initial treatment)	Graft survival (days) with same donor	Graft survival (days) with third-party donor
DA to PVG high-responder		
AR-C141990 (100 mg/kg)	>50	
AR-C148990 (30 mg/kg)	>50	
PVG to DA low responder		
CsA (5 mg/kg orally days 0–9)	>50 >50	7
AR-C141990 (30 mg/kg)	>50 >50 >50	

Toxicity

The body weights of all animals were measured on a daily basis. We calculated the mean proportional changes in body weight relative to pretransplantation levels. The changes in body weight in groups showing effective treatment for long-term graft survival and CsA are plotted in Fig. 2. Overall, CsA-treated animals lost most weight immediately after transplantation, compared to the other groups, and gained weight slowly thereafter. Animals treated with AR-C148990 gained weight rapidly after transplantation, and to a greater extent than other animals. Groups that received ineffective treatment, such as AR-C152593 and AR-C141990, had weight changes similar to those in the AR-C155187 group. None of the groups showed any signs of toxicity from the test agents.

Discussion

The compounds and associated experimental results given in this study build significantly on the results reported earlier. AR-C117977 and AR-C122982 produced prolonged graft survival in rodent models of cardiac transplantation. These two compounds are very closely related

Table 3. Associations between plasma concentrations, occupancy, and graft survival.

AR-C	K_d (ng/ml)[a]	B_{max} (Blood)[b]	Route	Dose (mg/kg)	Day	Mean (ng/ml)[c]	Occupancy (%)[d]	Comment
141990	40	380	sc	3	3	10	20	Similar to controls
				10	3	38	50	Maintained in dosing period
				30	3	136	77	Survival postdosing
				100 (50 b.i.d.)	3	55	58	
					12	185	82	Long-term survival
148990	80	350	sc	30 (15 b.i.d.)	3	365*[e]	64	
					6	519*	76	
					12	478*	73	Long-term survival
155187	2.6	320	sc	30	3	42	94	
					12	75	96	Long-term survival
				100	3	563	>99	
					12	361	>99	Long-term survival
152593	15	300	sc	10	3	1	6	Similar to controls
				30	3	4	21	
					6	69	82	
					12	15	50	Long-term survival
155858	2	350	p.o.	10	3	203*[f]	57	
					12	25*	7	Similar to controls
				30	3	278*	77	
					12	36*	10	Similar to controls
				100	3	337*	91	
					12	52*	15	Maintained in dosing period
			sc	100	3	244*	68	
					12	212*	60	Long-term survival

[a,b]Determined from plotting blood concentration against plasma concentration of compounds.

[c]Measured in plasma, except where indicated by an asterisk.

[d]Occupancy was calculated as reported previously where compound concentrations could be measured from plasma (%Occupancy = $100*[P]/(K_d + [P])$. Where samples from blood were used, plasma concentrations ($[P]$) were calculated using the formula

$$[P] = \frac{-(B_{max} + K_d - [\text{Blood}] + \text{SQRT}((B_{max} + K_d - [\text{Blood}]^2 - 4 * [\text{Blood}] * K_d)}{2}$$

Occupancy was then calculated as previously, using this calculated plasma concentration.

[e]Blood data used owing to analytical issues with plasma samples.

[f]Measured from blood, as plasma concentrations were below LOQ.

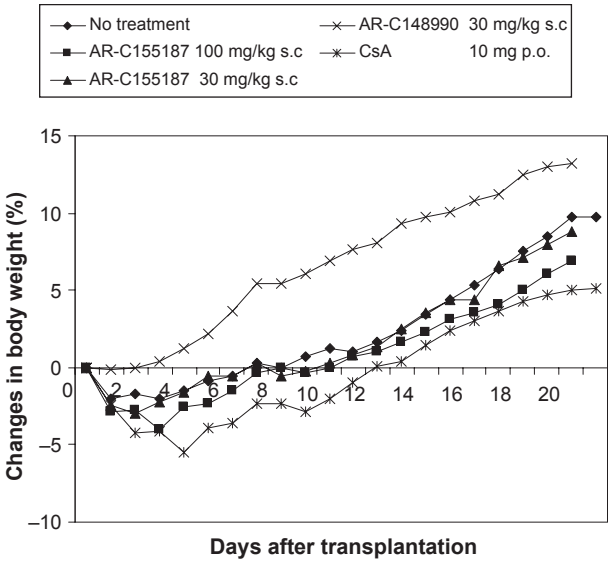

Figure 2 Changes in body weight from before transplantion (%), measured on a daily basis for 20 days after transplantation. AR-C148990 and AR-C-155187 were administered for 10 days by subcutaneous injection while cyclosporine A (CsA) was given orally for 40 days.

in both structure (Table S1) and physical properties. They have high lipophilicity and very low solubility, with very short PK half-lives and negligible oral bioavailability in rodents. They are also predicted to have high clearance and low oral bioavailability in man. Consequently, while they have drawn our attention to the potential usefulness of inhibitors of the MCT family as immunosuppressants, in themselves they are not good drug candidates. Whether or not it would be possible to move away from compounds with this type of structure to compounds with physical and PK properties more consistent with those of drug candidates was an important question to be addressed in this study.

The compounds tested in this study have good solubility and measurable oral bioavailability. They are also predicted to have improved PK properties in man relative to AR-C117977 and AR-C122982. Given the change in properties and chemical structure (Table S1) from the earlier reported compounds, it was important to determine whether the effects on alloimmune responses would be maintained, thereby strengthening the link between inhibition of MCT and immunosuppression. The effects of these compounds on the GvH response and in the rat heart allograft experiments were closely correlated with the plasma levels of the compounds *in vivo* when normalised by binding affinity K_d. In the GvH experiment, there was a significant correlation between the level of the compound and inhibition of the response, which was independent of the molecular structure of the compound. Likewise, in the rat cardiac graft experiments, the level of receptor occupancy during the dosing period was critical for graft survival. Experience with previously studied compounds had suggested that prolonged exposure to a compound beyond the 10-day dosing period was required for long-term graft survival, and this conclusion was further supported by the results with the present compounds.

The duration of graft survival in the high-responder cardiac transplants is best explained in the context of the plasma concentrations achieved with each compound (Table 3). In treatment groups where the minimum occupancy was low (e.g. AR-C141990 at 3 mg/kg s.c.), rejection occurred in the same time scale as untreated control animals. Where occupancy was high during the dosing period, but where levels of compound fell promptly after cessation of treatment (e.g. AR-C155858 100 mg/kg p.o.), the graft was maintained during the dosing period, but long-term graft survival was not achieved. It was only where minimum receptor occupancy remained ≥50% on day 12 (2 days following cessation of dosing) that long-term graft survival was observed (e.g. AR-C148990 30 mg/kg s.c. and AR-C155187 30 mg/kg s.c.).

Where the apparent (mean) MCT-1 receptor occupancy fell to around 50% on day 12, graft survival within the dose group exhibited high variability (e.g. AR-C152593 30 mg/kg sc) – perhaps reflecting sensitivity to interanimal PK variability at this exposure threshold. Overall, the correlation of long-term graft survival with continued high receptor occupancy, as was reported earlier [6] appears justified for this group of compounds. Detailed study of future compounds that match our criteria for progression as drug candidates would be useful to extend these conclusions further.

All the compounds examined to date inhibit MCT-2 as well as MCT-1. Occupancy of MCT-2 was estimated to be low during the course of most of the *in vivo* experiments, based on the potency of compound binding in a radio-ligand binding assay using MCT-2 expressed in yeast membranes. However, it has recently been shown that the potency of interaction of this class of compounds, exemplified by AR-C155858, can be modulated by the presence of chaperone proteins [14]. Although the present studies clearly demonstrate the primary role of MCT-1 blockade in immunosuppression, the significance of MCT-2 inhibition for graft survival will only become clear once more selective MCT-1 or MCT-2 inhibitors are available.

In summary, we have presented a number of new analogues that inhibit MCT-1 and that have immunosuppressive properties in rat cardiac graft recipients and in GvH experiments. No overt signs of toxicity were seen in the ani-

mal models. Level of efficacy correlated with plasma concentration and level of MCT-1 inhibition. The compounds studied cannot be regarded as drug candidates because of low predicted oral bioavailability in man. However, the findings in these experiments have taken us further towards defining the PK properties that may help us to identify such candidates among inhibitors of MCT-1.

Authorship

CP, ZQ, DF: participated in research design, performance of experiments, data analysis and writing of the paper. CMM, RVB, DKD, HE: participated in research design, data analysis and writing of the paper.

Funding

The authors have declared no funding.

Acknowledgements

We thank Elain Holness and Bob Craggs for excellent experimental assistance in the GvH studies.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Chemical structures.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

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