Pharmacokinetic Advantage of Intra-arterial Cyclosporin A Delivery to Vascularly Isolated Rabbit Forelimb. I. Model Development

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ABSTRACT

Effective antirejection therapy with minimal systemic morbidity is required if limb transplantation is to become a clinical reality. We investigated whether i.a. infusion of cyclosporin A (CSA) into the vascularly isolated rabbit forelimb will distribute drug homogeneously to the tissues and produce higher local drug levels than same-dose i.v. treatment, thereby improving the therapeutic index. CSA 4.0 mg/kg/day was infused continuously via osmotic minipump into either the right brachial artery (i.a. group) or jugular vein (i.v. group) of New Zealand rabbits. Ligation of all muscles at the right mid-arm level was performed in the i.a. group to eliminate collateral circulation and simulate allografting, while leaving bone and neurovasculature intact. On day 6, CSA concentrations were measured in skin, muscle, bone, and bone marrow samples taken from different compartments of the right and left forearms in the i.a. group and right forearm only in the i.v. group. There were no significant differences between compartmental CSA levels in all tissues examined on the locally treated, right side during i.a. infusion, indicating that drug streaming from the catheter tip is not occurring in our model. During i.a. infusion, mean CSA concentrations were 4- to 7-fold higher in the right limb than in the left limb in all four tissues examined. Tissue CSA levels in the left limb were equivalent to those achieved during i.v. infusion, but CSA concentrations in blood, kidney, and liver were higher during i.a. infusion. These favorable, preliminary, single-dose pharmacokinetic results warrant further investigation in our novel rabbit model.

Composite tissue allografts (CTAs) are modules composed of various combinations of skin, s.c. tissue, connective tissue, muscle, bone, cartilage, bone marrow, and neurovascular tissue. These tissue blocks have tremendous potential clinical application for precise functional and structural reconstruction of major acquired and congenital peripheral tissue defects. Previous electrophysiologic, histologic, and functional studies conducted in rat (Black et al., 1988; Yu et al., 1989), canine (Aebi et al., 1986; Doi et al., 1989), and primate (Daniel et al., 1986; Stark et al., 1987; Bain et al., 1989; Stevens et al., 1990) models have repeatedly demonstrated that the degree of nerve regeneration and overall neuromuscular and physical performance achievable in CTAs is not significantly different from that observed in syngeneic controls, as long as high-dose, continuous cyclosporin A (CSA)-based therapy is maintained to prevent rejection across major histocompatibility barriers. Therefore, it appears that the single most important obstacle currently preventing limb transplantation from becoming a clinical reality is not the inability to restore function, but rather the lack of specific, safe, and effective immunosuppressive therapy. Although chronic systemic administration of relatively high doses of nonspecific immunosuppressive agents is readily accepted in the visceral organ transplant recipient faced with poor quality of life or death, this would at present be unacceptable in the patient requiring musculoskeletal reconstruction (Paskert et al., 1987).

One approach toward reducing the drug-specific and general adverse consequences of systemic immunosuppression in CTA recipients, and thereby improving the clinical feasibility of the procedure, is the use of local drug administration systems. Locoregional drug delivery may establish a more selective presence of currently available nonspecific immunosuppressive agents in the transplanted limb or limb com-

Abbreviations: $C_{\text{systemic}}$(i.a.), systemic drug concentration at steady state during i.a. infusion; $Cl_s$, systemic clearance; CSA, cyclosporin A; CTA, composite tissue allograft; EMIT, enzyme-multiplied immunoassay; inf, constant drug infusion rate; $Q_T$, target organ blood flow.

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ponent, with a concomitant reduction in systemic drug exposure (Gruber, 1992). During the past decade, favorable experiences with pump-based local immunosuppressive therapy have been reported in multiple rat and canine visceral solid-organ and cellular allograft models by our laboratory and others (Gruber, 1996). Coincident with this work, several investigators have demonstrated long-term survival of rodent skin allografts treated topically with CSA (Lai et al., 1987; Black et al., 1990; Sönmez et al., 1991), combination corticosteroid and CSA (Llull et al., 1995), or tacrolimus (Fujita et al., 1997). In light of these developments, the goal of our research was to develop a rabbit forelimb model to investigate the pharmacokinetic parameters of i.a. drug delivery to the extremity while simulating the conditions existing following composite tissue allografting. We then used the model to determine 1) homogeneity of drug distribution within limb tissues; 2) regional pharmacokinetic advantage; 3) limb tissue drug concentration ratios; and 4) generalized tissue distribution of drug during local versus systemic, single-dose, CSA infusion.

Materials and Methods

Animals. Seventeen outbred male New Zealand rabbits, 2.8 to 3.0 kg in weight, were used in our studies and cared for in accordance with guidelines established by the Institutional Animal Care and Use Committee of the University of Louisville School of Medicine. Rabbits were housed in separate cages at constant room temperature with a 12-h light/dark cycle and maintained on a balanced rodent diet with free access to water throughout the experiment.

Measurement of Tissue Residual Blood Content. When determining tissue levels of CSA in solid organs, it is necessary to correct for the variable amount of residual blood present to ensure that what is being measured accurately reflects the drug concentration present in the tissue itself (Bernareggi and Rowland, 1991). Therefore, in a preliminary study, we measured tissue residual blood content in three rabbits using $^{51}$Cr-sulfate red cell tagging as described by Bernareggi and Rowland (1991) for the rat. Briefly, 15 ml blood was drawn from each animal into sodium citrate anticoagulant tubes and centrifuged at 1500 $g$ for 15 min. Plasma was separated and the cells washed three times with isotonic saline and then incubated with 300 $\mu$Ci $^{51}$Cr for 2 h at 37°C. The excess $^{51}$Cr was removed by successive washing with saline and the labeled cells reinfused into the animal. After 30 min, animals were euthanized by bleeding and blood and tissue samples from skin, muscle, bone marrow, fat, heart, lung, liver, and kidney were obtained. Tissue-associated radioactivity was measured using the enzyme-multiplied immunoassay (EMIT) specific method (Bernareggi and Rowland, 1991). The heart, right lobe of the liver, right kidney, and lower lobe of the right lung were removed from all animals for determination of CSA concentration. Samples of the following forelimb tissues were obtained for determination of CSA levels from both right and left sides in the i.a.-treated animals and from the right side only in animals receiving systemic therapy: dorsal (posterior) skin, posterior muscles of the extensor compartment, ventral (anterior) skin, anterior muscles of the flexor compartment, ulna, and radius. Bone marrow was harvested from the ulna bilaterally only in animals receiving local CSA infusion. All samples were stored at −80°C until analysis.

CSA Assay. Whole-blood CSA concentrations were determined using the enzyme-multiplied immunoassay (EMIT) specific method (Beresini et al., 1993) on the Cobas Mira chemistry analyzer. Specimens were collected in EDTA, mixed well, and an aliquot frozen in

Pump/Catheter Preparation. Alzet 2 ML1 miniosmotic pumps (Alza Corporation, Palo Alto, CA) were used for continuous i.a. and i.v. drug administration. These pumps have a reservoir volume of 2 ml, and are designed to deliver at a fixed rate of 10.0 $\mu$l/h for 1 week. A composite catheter was fashioned for vessel implantation, consisting of a 4-cm proximal segment of Intramedic PE-60 tubing (A. 0.048 in; Clay Adams, Parsippany, NJ) connected to the pump delivery portal and glued to a 25-cm distal segment of Intramedic PE-10 (A. 0.024 in) tubing. Two small beveled pieces of PE-60 tubing were passed over and glued to the tip of the PE-10 tubing, serving as attachment points for ligatures securing the catheter tip in the vessel. Pumps were filled with CSA solution (Sandimmune, 50 mg/ml; Sandoz Pharmaceuticals Corp., East Hanover, NJ) and primed by placing the pump/catheter assembly in sterile normal saline solution for 24 h before implantation.

Rabbit Forelimb Model of Local Immunosuppression. Animals were anesthetized with an i.m. injection of ketamine (37.5 mg/kg) and xylazine (5 mg/kg). A circumferential skin incision was made around the right mid-arm. All muscle groups were interrupted at this level with 0-silk ligatures to eliminate collateral circulation and simulate allografting. The brachial artery was dissected free from surrounding tissues and, proceeding in a proximal direction, all its branches in the arm were ligated with 6–0 silk suture up to the level of the thoracodorsal artery. The thoracodorsal artery was then dissected free for at least 1 cm and ligated distally. The distal end of the previously prepared infusion catheter was inserted into the thoracodorsal artery stump and advanced proximally until its tip reached, but did not enter, the thoracodorsal/brachial artery junction. The catheter was secured in place with 6–0 silk ties. The pump attached to the other end of the catheter was placed in a s.c. pocket overlying the serratus anterior muscle. Figure 1 schematically depicts the location of the arterial pump/catheter system at the conclusion of the procedure. In animals receiving i.v. therapy, circumferential skin incision with ligation of muscle bundles was not performed on either forelimb. Instead, the same infusion catheter was placed in the right external jugular vein with the pump in a subcutaneous pocket.

Pharmacokinetic Study. Seven rabbits received CSA 4.0 mg/kg/day by continuous i.a. infusion and seven rabbits received the same dose by continuous i.v. infusion. On postoperative day 6, animals were anesthetized with ketamine and xylazine as described above. Following left thoracotomy, a blood sample was drawn from the aorta for determination of whole-blood CSA concentration, the aorta was transected, and the animal euthanized by bleeding, which causes peripheral vasoconstriction and results in minimal residual blood in the tissues (Bernareggi and Rowland, 1991). The heart, right lobe of the liver, right kidney, and lower lobe of the right lung were removed from all animals for determination of CSA concentration. Samples of the following forelimb tissues were obtained for determination of CSA levels from both right and left sides in the i.a.-treated animals and from the right side only in animals receiving systemic therapy: dorsal (posterior) skin, posterior muscles of the extensor compartment, ventral (anterior) skin, anterior muscles of the flexor compartment, ulna, and radius. Bone marrow was harvested from the ulna bilaterally only in animals receiving local CSA infusion. All samples were stored at −80°C until analysis.

CSA Assay. Whole-blood CSA concentrations were determined using the enzyme-multiplied immunoassay (EMIT) specific method (Beresini et al., 1993) on the Cobas Mira chemistry analyzer. Specimens were collected in EDTA, mixed well, and an aliquot frozen in

Fig. 1. Location of pump/catheter system in vascularly isolated rabbit forelimb model of local immunosuppression.

Vol. 289
1186 Shirbacheh et al.

Brachial Artery

Thoracodorsal Artery

Local (intra-arterial)
Catheter and Pump Assembly

Ligation of
Collateral Circulation
a dry ice/ethanol bath. After thawing and mixing to ensure homogeneity, 200-μl aliquots were extracted with 400 μl methanol (reagent grade), vortexed, and then centrifuged before analysis against a stored standard curve. Run acceptability was determined by whole blood controls that were run at the beginning and end of each specimen batch. Those specimens with values exceeding the upper limit of the standard curve (500 ng/ml) were diluted 1:1 with blank whole blood and re-extracted along with an elevated control.

Tissue specimens were extracted into methanol before analysis for CSA. Tissue was weighed, placed in a labeled polypropylene tube, and ice-cold methanol (reagent grade) added to give between 50 and 100 mg tissue/ml methanol. The extraction tubes were placed in ice and the contents homogenized using the PowerGen Homogenizer (Fischer Scientific, Pittsburgh, PA) at 25,000 rpm for 10 to 15 s or until the tissue was totally fragmented. Each tube was capped and frozen at −70°C until analysis. Just before analysis, each extract was thawed, well mixed, transferred to a 2-ml microtube, and centrifuged at 10,000g for 4 min. Four hundred microliters of each methanol supernatant was added to 200 μl deionized water, vortexed, and centrifuged before analysis for CSA using the EMIT assay. Controls were assayed with each batch to determine run acceptability. Tissue extracts with CSA concentrations greater than 500 ng/ml were diluted appropriately with methanol until they were within the assay range. Values were expressed as nanograms of CSA per gram of tissue. Ninety-seven to 100% CSA recovery was obtained in all tissues examined over the concentration range 100 to 10,000 ng/g tissue, and recovery studies demonstrated that both skin and muscle tissue did not interfere with the EMIT assay.

Data Analysis. Compartmental tissue CSA concentrations were compared on both right and left sides of i.a.-treated animals using a paired Student’s t test. Right and left forearm mean tissue CSA levels as well as mean skin/muscle, skin/bone, and bone marrow/skin CSA concentration ratios were compared in locally treated rabbits using a paired t test. Finally, right and left forearm mean tissue CSA concentrations and concentration ratios in the group receiving i.a. infusion were compared with those obtained from right forearm tissues in the group receiving i.v. infusion using an unpaired t test. All values are expressed as mean ± S.E. p ≤ .05 was regarded as statistically significant.

Results

Measurement of Tissue Residual Blood Content. The residual blood content for various rabbit tissues is given in Table 1. In general, little variability was observed among the animals. It is noteworthy that, with the exception of bone marrow (0.05 ml blood/g tissue), forearm tissue components relevant to our model have a residual blood content (range 0.004–0.008 ml blood/g tissue) that is in general an order of magnitude lower than that present in visceral solid organs such as the heart, lung, liver, and kidney (range 0.05–0.23 ml blood/g tissue). Therefore, measured CSA concentrations in forelimb skin, muscle, and bone are truly reflective of what is in the tissue, are not significantly altered by residual blood ( <1%), and did not require correction. In contrast, measured CSA concentrations in bone marrow, heart, lung, liver, and kidney, tissues with residual blood content ranging from 5 to 23%, were corrected for the contribution of blood.

Homogeneity of Drug Distribution Within Limb Tissues. Figure 2 depicts the skin and muscle CSA concentrations in the anterior and posterior compartments and bone CSA concentrations in the radius and ulna of the right (R) and left (L) forearms during i.a. infusion and in the R forearm during i.v. infusion on day 6. As expected, there were no significant differences between compartmental tissue drug levels within limbs exposed to systemic therapy (i.v.-R) or its equivalent (i.a.-L). However, the absence of significant differences in compartmental tissue drug concentrations within the limb receiving direct CSA infusion (i.a.-R) indicates that drug streaming from the catheter tip is not an important consideration in our model and that adequate mixing of drug in the bloodstream is occurring before the first bifurcation of the distal arterial tree resulting in homogeneous drug distribution.

Pharmacokinetic Advantage of Local CSA Infusion. Because no significant differences were noted with regard to compartmental skin, muscle, or bone CSA concentrations
within the locally or systemically treated forelimb, anterior/posterior and radius/ulna CSA levels were averaged to yield one mean CSA concentration value in each forelimb for each of the three tissues. Figure 3 demonstrates that in the i.a. infusion group, day 6 mean tissue CSA concentrations were significantly higher in the locally treated R limb than in the L limb ($p < .005$ in all cases), with the R/L ratio ranging from 4- to 7-fold. Tissue CSA concentrations in the L limb (i.a. — L) were equivalent to those achieved during i.v. infusion (i.v. — R) ($p = \text{N.S.}$ in all cases). Figure 4 illustrates that consistent 2.5:1 skin/muscle and skin/bone forearm CSA concentration ratios were maintained bilaterally during i.a. infusion as well as during i.v. infusion ($p = \text{N.S.}$ for pairwise comparisons in both cases). Similarly, the bone marrow/skin CSA concentration ratio was not significantly different between the R and L sides in the i.a. infusion group (12.8 ± 2.2 versus $8.9 \pm 0.9$, $p = \text{N.S.}$).

**Systemic Tissue CSA Levels During i.a. Versus i.v. Infusion.** Figure 5 compares the generalized tissue distribution of CSA on day 6 in the i.a. and i.v. infusion groups. In the i.a. group, skin, muscle, and bone CSA concentrations are depicted for the L (systemically treated) forelimb. When compared with same-dose i.v. infusion, mean systemic tissue CSA concentrations during i.a. infusion were minimally increased in skin, muscle, bone, and lung (i.a./i.v. mean concentration ratios ranging from 1.08–1.29, $p = \text{NS}$); moderately increased in heart (i.a./i.v. = 1.50; $p = 0.09$); and significantly increased in blood, kidney, and liver (i.a./i.v. ratios ranging from 1.65–1.84; $p < 0.05$).

**Discussion**

We developed a novel rabbit forelimb model using a pump/catheter infusion system to investigate the pharmacokinetics of i.a. immunosuppressive drug delivery to the extremity while simulating the conditions existing after composite tissue allografting. Interruption of collateral circulation through the muscle bundles at mid-arm level and ligation of all side branches made the brachial artery the sole source of blood flow to the distal extremity while leaving the bone and vasculature intact, thus obviating the need for microsurgery and bony reconstruction. In addition, by not interfering with forelimb innervation, postoperative morbidity was significantly reduced, with the animals possessing intact sensation and able to ambulate and feed comfortably.

The potential for drug streaming from the infusion site is an important pharmacokinetic pitfall that must be addressed in any model of i.a. drug administration (Dedrick, 1988). If inadequate mixing of drug occurs before the first distal arterial bifurcation, heterogeneities may persist deep into the tissue through a number of branch levels, with portions of the target region receiving a suboptimal dose of drug and other areas receiving toxic levels. Blacklock et al. (1986) and Saris et al. (1988) noted profound maldistribution of iodoantipyrine tracer following internal carotid artery infusion in rhesus monkey and rat brain, respectively, and frequent evidence of both central nervous system and ocular toxicity has been noted during carotid artery infusions of chemotherapeutic agents for i.c. tumors (Stewart et al., 1982; Lehane et al., 1983; Greenberg et al., 1984; Hochberg et al., 1985). In addition, inhomogeneous drug distribution may lead to a localized saturation of both drug uptake and/or elimination mechanisms within the target organ (Dedrick, 1986, 1988; Blacklock et al., 1986; Daeman, 1987). Fortunately, we found no evidence of significant maldistribution of drug in any of
the three tissue components examined (skin, muscle, and bone) during i.a. infusion in our rabbit forelimb model.

There are two kinds of pharmacokinetic advantage of i.a. over i.v. drug delivery. The regional advantage reflects the degree to which drug concentration in the target organ can be increased by giving the drug locally rather than systemically, and is given by the expression $1 + Cl_s/Q_T$, where $Cl_s$ is the systemic clearance of drug outside the target organ calculated during i.v. administration, and $Q_T$ is blood flow to the target organ (Collins, 1984). Regional advantage is established during the first passage of drug through the target organ, because the drug then returns to the systemic circulation and is distributed as although injected i.v. (Eckman et al., 1974; Collins, 1984). The systemic advantage reflects the degree to which drug delivery to the systemic circulation can be reduced by local infusion via the first-pass elimination or metabolism of drug by the target organ, and increases as the extraction ratio of drug by the target organ increases.

Therefore, different organs differ in their suitability for regional drug delivery depending on their blood flow, their capacity for drug elimination, and the pharmacokinetics of the agent chosen for administration (Daeman, 1987). Because little or no metabolism of CSA occurs within limb tissues, extremity infusion would not be expected to produce a systemic advantage. However, the relatively low blood flow to the upper extremity would be expected to produce a considerable regional pharmacokinetic advantage of i.a. CSA infusion not achievable in other high-flow organs such as the liver or kidney, as long as tissue blood flow was low relative to drug clearance. Along these lines, we were able to demonstrate a 4- to 7-fold regional advantage of local CSA administration, as determined from the R/L ratio of drug concentrations, in all four limb tissues examined at the single dose chosen for study.

Both the equation given above and the R/L ratio used in this study for calculation of regional advantage are only valid after steady state has been reached (Smits and Thijssen, 1987). Along these lines, Awni and Sawchuk (1985) examined the pharmacokinetics of CSA in the male New Zealand White rabbit following i.v. bolus and constant-rate infusion at several doses. They reported mean CSA elimination half-lives in the 3- to 4-h range in their i.v. bolus study, and found that during continuous i.v. infusion, steady-state CSA levels were reached in the blood within 36 h in each animal at each of three dose levels, ranging from 8 to 32 mg/kg/day. Moreover, Bernareggi and Rowland (1991) noted that steady state was achieved in whole blood within 3 to 4 days following the initiation of pump-based, continuous s.c. CSA infusion in rodents, and their allometric predictions for CSA concentrations in various human tissue compartments indicate that steady state is reached in skin, muscle, and bone by 72 h postinfusion. Therefore, although we did not directly address this issue in the present study, previous work by others suggests that steady state was reached in our model in whole blood as well as in each limb tissue component by the day 6 endpoint.

However, it is somewhat difficult to explain our paradoxical observation that mean whole-blood CSA levels were 1.7-fold higher in the i.a. infusion group than in the i.v. infusion group. On the contrary, one would expect that steady-state systemic CSA levels produced during i.a. infusion would at most equal those produced during i.v. infusion, assuming no first-pass extraction of drug in the forelimb. One possible explanation is that while passing through the locally infused, low-flow rabbit forelimb, red blood cells are transiently exposed to high concentrations of CSA and become “loaded” with drug. This additional drug is then carried into the systemic circulation and is preferentially distributed to certain tissues (heart, kidney, and liver) based on steady-state tissue/whole blood equilibrium distribution ratios (Bernareggi and Rowland, 1991). The steady-state CSA concentration in the brachial artery during i.a. infusion is given by the sum of two components: $inf/Q_T + C_{systemic(i.a.)}$, where $inf$ is the constant infusion rate of drug and $C_{systemic(i.a.)}$ is the concentration of drug present in the blood returning to the limb at steady state (Collins, 1984). We have previously determined brachial artery blood flow to be 2.3 ± 0.1 ml/min by electromagnetic probe measurement in 18 healthy male New Zealand rabbits of the same weight as those used in our study. Using our measured mean value of 293 ng/ml for $C_{systemic(i.a.)}$, with $inf = 12$ mg/day and $Q_T = 2.3$ ml/min, yields an estimated local CSA concentration of 3900 ng/ml. Because saturation of erythrocytes with CSA does not occur until concentrations above 4000 to 5000 ng/ml are reached (Atkinson et al., 1983; Niederberger et al., 1983), this calculation is consistent with our “loading” hypothesis. Further studies comparing steady-state whole-blood CSA levels during i.v. and i.a. infusion at multiple doses, as well as examining the distribution of CSA within whole blood locally and systemically during local drug administration, are necessary to substantiate our observation and elucidate the mechanism(s) for it.

To our knowledge, the generalized tissue distribution of CSA within rabbit tissues following i.v. bolus or during continuous i.v. administration has not been previously reported. Our findings are similar to those observed in the rat by Bernareggi and Rowland (1991) following 6 days of continuous, pump-based s.c. CSA infusion at 2.7 mg/kg/day. In this study, the lowest parent drug concentrations (measured by high-performance liquid chromatography) were found in muscle; intermediate concentrations in skin, bone, and heart; and highest concentrations in fat, liver, spleen, lung, thymus, and pancreas. Interestingly, skin/muscle and skin/bone CSA concentration ratios were 3.0 and 1.6, respectively, similar to our values of 2.5:1. Moreover, our results in the rabbit regarding residual organ blood content following exsanguination were also similar to those demonstrated by these authors in the rat. They found lowest residual volumes of blood per gram of tissue in eye, skin, fat, and muscle; intermediate volumes in bone, kidney, heart, and liver; and highest volumes in lung and spleen.

Correlation of transplant, systemic tissue, and whole-blood levels with antirejection efficacy in previous experimental studies of local immunosuppression, as well as in liver transplant recipients, suggests that maintenance of high local/systemic tissue concentration gradients during extremity CSA infusion may be useful in preventing composite tissue allograft rejection with decreased systemic drug exposure. In a dual transplant model, Black et al. (1990) applied topical CSA to one of two rat skin allografts on the same animal following a 10-day course of systemic CSA treatment. Allograft survival was significantly prolonged and mean graft tissue CSA levels were 23-fold greater (37.5 versus 1.6 μg/g) in grafts receiving topical CSA when compared with vehicle-
treated controls, even though both grafts were exposed to the same subtherapeutic systemic serum CSA levels. Along these lines, Stepkowski et al. (1989) found that continuous i.a. infusion of low-dose CSA significantly prolonged rat hetero-
topic heart allograft survival when compared with same-dose i.v. administration. Steady-state tissue CSA levels in recipi-
ent native heart, kidney, spleen, liver, muscle, and blood on day 6 postgrafting were equivalent in systemically and lo-
 tally treated animals, but CSA levels in the heart graft were 9-fold higher in the latter group. Finally, Sandborn et al. (1992) demonstrated that hepatic tissue, but not blood, CSA concentrations correlated with early cellular rejection in liver transplant patients.

In summary, we developed a novel rabbit forelimb model to study the pharmacokinetics of i.a. immunosuppressive drug delivery to the extremity while simulating the conditions existing following composite tissue allografting and leaving bone and neurovasculature intact. In the locally treated limb, CSA was distributed homogeneously within tissue compo-
nents and mean tissue drug levels were 4- to 7-fold higher than those in the contralateral limb or in the limbs of ani-
mals receiving same-dose i.v. therapy. Despite being exposed to 10-fold higher steady-state whole-blood CSA levels, tissue concentration ratios in the locally treated limb were equiva-
 lent to those on the opposite side, indicating that a predict-
able 10-fold higher steady-state whole-blood CSA levels, tissue concen-
trations correlated with early cellular rejection in liver transplant patients.

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