A Rat Model Designed for the Continuous Intraarterial Infusion of Cyclosporine

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ABSTRACT

Background. Limb allotransplantation is not a life-saving treatment. However, large doses of immunosuppressive agents are needed. There is an urgent need to increase the selectivity and targeting of drugs.

Methods. We designed a rat model for intraarterial infusion of cyclosporine (CSA) based on the hindlimb replanted model to simulate the limb allotransplantation. To investigate whether intraartery infusion could improve the drug's distribution, we infused CSA 4.0 mg/kg per day continuously into either the superficial epigastric artery (IA group) or superficial epigastric vein (IV group) of Lewis rats.

Results. On day 10, CSA concentrations were measured in skin, muscle, and bone tissues of hindlimb. Samples were taken from different parts of the bilateral hindlimbs in the IA group and right hindlimb only in the IV group. Tissue concentrations of the perfusion side were much higher in IA group. Systemic concentrations of IA group were higher than IV group.

Conclusions. These results warrant further research in our next limb allotransplantation model.

LIMB ALLOTRANSPLANTATION is different from organ transplantation, because the limb is a composite tissue that contains the most antigenic tissues such as skin, muscle, and bone [1]. Conventional immunosuppressive therapy of organ transplantation cannot completely avoid the acute and chronic rejection of limb grafts, so we need to increase the dosage of immunosuppressive agents. However, large doses of immunosuppressive agents seriously damage the immune system and cause the infection, liver and kidney damage, hypertension, diabetes, nerve damage, secondary tumors, and even death [2].

Limb transplantation has caused a fiercely ethical debate [3]. Is it desirable to take a life-threatening risk for the reconstruction of a limb's function and form? Outcomes of the debate are that limb allotransplantation should have a very high "benefit–cost" ratio. To achieve this aim, new immunosuppressive agents need to be developed, and we should also increase the selectivity and targeting of the conventional immunosuppressive agents [4,5]. By direct infusion of drugs from the nutrient artery to the allograft, we can increase the concentration in the local tissue and reduce the systemic concentration to avoid systemic toxicity. The pharmacokinetics of local immunosuppression have been demonstrated by many researchers. In 1988, Ruers et al [6] infused budesonide into the anastomotic artery of a rat's cardiac allograft using an osmotic pump. Drug absorption of the heart tissue was very rapid, and the drug concentration of the myocardium was 29.6 ng/mg, whereas the drug concentration of systemic tissue was only 0.34 ng/mL. Shirbacheh et al [7] designed a rabbit model of isolated forelimb to simulate the blood supply of limb transplantation, and infused cyclosporine (CSA) through

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the brachial artery. Their results demonstrated that the tissue concentration of infused limb was much higher than in the noninfused limb. The infused limb has a very high odds ratio compared with the noninfused limb [7].

The model of limb allotransplantation usually used Lewis rat as recipients, but there was no report on the Lewis rat model of intraarterial infusion [8–10]. We have designed a novel Lewis rat model of intraarterial infusion to simulate limb allotransplantation. By infusing CSA, which is one of most commonly used drugs in our animal model, we try to prove the feasibility and stability of our animal model and provide a novel model for researchers to study the effect of a local immunosuppressant.

MATERIALS AND METHODS

Animals

Sixty specific pathogen–free male Lewis rats (RT1[l]; Vital River, Beijing, China.) aged 8–10 weeks and weighing 200–250 g were used in our studies. All procedures were approved by the Experimental Animals Committee of the Fourth Military Medical University. The disposal of experimental animals complied strictly with the requirements of experimental animals. Veterinary care of the animals was provided by the specific pathogen–free animal facility of the Fourth Military Medical University.

Operative Procedure

Longer LSP10-1B infusion pumps (Longer Corporation, Bao Ding, China) were used for continuously intraarterial (IA) and intravenous (IV) drug administration. These pumps were designed to deliver drug uninterruptedly at a constant speed. Rats were anesthetized with pentobarbital sodium (40 mg/kg) by intraperitoneal injection. A circumferential skin incision was made around the midfemoral level of the right hindlimb. The femoral artery was dissected from surrounding tissues and all its branches were ligated with 10-0 nylon except the superficial epigastric artery. The replantation model of the rat hindlimb was done as previously described [11]. Femoral vessels were cut off at the distal level of the superficial epigastric artery. The bone, muscles, and sciatic nerve were amputated at the midfemoral level. The femoral vessels were anastomosed microsurgically with 10-0 nylon and the sciatic nerve was sutured epineurally with 8-0 nylon. Femoral osteosynthesis was performed using an 18-gauge needle as an intermedullary rod. An arterial catheter (RFA-04; SAI, Lindenhurst, IL) with a diameter of 0.67 mm and 46 cm long was designed for vessel implantation consisting of a Preclinical Mini-Port by Smiths (Fig 1A). The distal end of the catheter was inserted into the superficial epigastric artery of the right limb until the tip reached the femoral artery. The catheter was secured with 10-0 nylon (Fig 1B). Then the skin was sutured with 4-0 silk. The Mini-Port was embedded in the subcutaneous tissue of the back and attached to the SAI Quick Connect Systems designed for rat (SAI) consisting of harness, swivel, tether, and swivel mount (Fig 1C). The rat could not bite the catheter or the hindlimb with this harness. The SAI Quick Connect System was connected to the infusion pump. The infusion system was filled with CSA solution (Sandimmune, 50 mg/mL; Sandoz Pharmaceuticals Corp., East Hanover, NJ). Every rat was housed in a separate cage and allowed normal activity without restriction. In the IV group, the same operation was performed on right hindlimb, except that the infusion catheter was placed in the superficial epigastric vein.

Specimen Collection

Thirty rats received CSA 4.0 mg/kg per day by continuous IA infusion and 6 rats received the same dose by continuous IV infusion. On postoperative days 2, 4, 6, and 8, 6 animals in the IA group were anesthetized as described. A blood sample was taken from the aorta for determination of whole blood CSA concentration, and the animal was euthanized by bleeding to get minimal residual blood in the tissues. Skin biopsies of right and left hindlimbs were obtained for determination of tissue CSA concentration. On postoperative day 10, the blood, heart, liver, kidney, and lung tissues as well as the skin, muscle, and bone tissues from right and left hindlimbs of both groups were obtained for determination of CSA concentration. All samples were stored at −70°C until analysis.

CSA Concentrations Assay

CSA concentrations were determined using the enzyme-multiplied immunoassay (EMIT) specific method on the Cobas Mira chemistry analyzer [12]. Briefly, whole blood samples were collected and mixed ethylenediaminetetraacetic acid to ensure homogeneity, and then were extracted with methanol and centrifuged before analysis against a stored standard curve. Tissue samples were weighed and

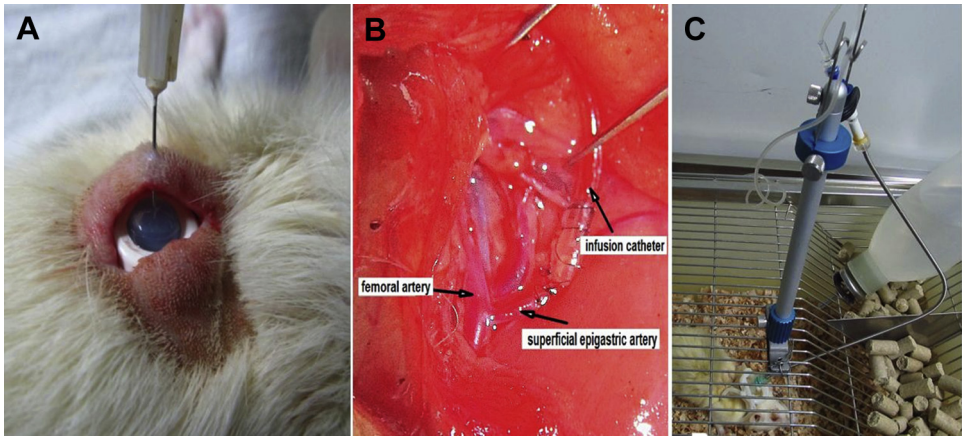

Fig 1. Operative procedure. (**A**) A mini-port was embedded in the subcutaneous tissue of the back. (**B**) A catheter was inserted into the superficial epigastric artery of the right limb. (**C**) The SAI Quick Connect Systems designed for rat.