行政院國家科學委員會專題研究計畫成果報告

異體肢體移植之犬臨床研究模式

Limb allotransplantation in dogs

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一、中文摘要

肢體移植因為是屬於複合組織移植, 其包括的組織形態和抗原變異性更加複雜, 在單一組織現今已有部分移植成功的例子;但在肢體移植上的成就仍有限, 有鑑於最近發展出的幾種新的免疫抑制劑皆有良好的效果，再加上顯微移植手術技術的進步, 於是本計劃以肢體缺損病犬為材料, 採用一隻意外斷肢超過三年之七歲混種母犬，進行異體肢體移植。肢體捐贈者為一兩歲公犬, 移植肢體除術後二週之水腫外，在免疫抑制劑 leflunomide 及 FK-506 之併用下，維持良好存活, 移植肢體循環及被毛生長良好，於手術後 35 日開始出現表皮感覺恢復現象, 本研究室擬就肢體功能之恢復，作長期觀察，並且使用 Y chromosome PCR detection 之方式，了解接受者體內細胞交移狀況, 進一步監視免疫耐性或移植物宿主病之發生情形, 進一步做為人類肢體移植之參考依據。

關鍵詞：肢體移植，移植，犬

Abstract

Limb allotransplantation has been a major challenge for surgeons because of the involvement of multiple antigenic tissues. This make limb transplantation still in the experimental stage while solid organ transplantations have long been a clinical routine. Since 1990, some novel immunosuppressive agents have been developed with better specificity and less toxicity. In a clinical canine patient with forelimb amputation for over 3 years, limb allo-transplantation was performed with leflunomide and FK506 as immunosuppressants. The transplanted limb survived the transplantation, with edematous swelling for 2 weeks after the surgery. Hair of the limb was growing back satisfactorily and rejection was controlled with the combination agents. Sensory recovery was first noted in 35 days after the transplantation. The animal is still under close monitor for functional recovery and late rejection. Y-Chromosome PCR will be used to detect chimerism of the patient to decide if tolerance or graft-versus-host disease can be predicted . This veterinary clinical model can be important reference for human limb allo-transplantation.

Keywords: limb allotransplantation, transplantation, dog

二、緣由與目的

Replacement prosthesis and prefabricated tissue reconstruction has not been able to fulfill the need of delicate human hands. Limb allotransplantation is the only possibility to rebuilt a full functional and esthetic limb. Organ transplantation has been a clinical reality for more than two decades. But limb transplantation was still in the stage of animal experiment because of complex tissue composition, skin and muscle antigenicity, and the inclusion of donor bone marrow cells.

In 1961, parabiosis was used by

Schwind to develop the tolerance to transplanted limbs on rats, however, 2/3 of the animals died of complications. Following that, goldwyn and Lance have tried immunosuppressive agents , antilymphocyte serum , and steroids in the attempt to control the rejection of limb grafts, however, the toxicity was too intense and many tissues were rejected due to insufficient dosage (16).

The antigenicity of limb tissues are so high that some researchers tried to reduce it by "selective transplantation". Which, by removing the most antigenic tissues including the skin, may delay graft rejection. But the method is not clinically practical (1,3,12,17,24,32,35,37,41). In 1976, the introduction of cyclosporine improved the survival of solid organ transplantations. Many study of limb allotransplantation had been tried on small animal models. The results of rabbit and baboon models have not been very satisfactory. But the rat model has more optimistic results. (12,15,27)

In the rat model, F1 hybride to parent model had been tried on LBN to LEW rats. Cyclosporine successfully prevented the limb rejection as long as the treatment going on. Composite tissue transplantation in the BN to LEW may also survive under cyclosporine 15mg/kg/d IM. However, the dosage maintaining composite tissues or limb has been very high that routine dosage maintaining solid organ will not prevent limb rejection. (5,6,8,19,23,25,26,33,38,40)

In the past few years, a number of novel immunosuppressive drugs have been developed with better specificity and less toxicity. Which render the research of limb allotransplantation becoming more optimistic. However, most trials in the rat model still needed very high dose of immunosuppression , and the functional recovery was far from satisfactory. It is very important to find a immune modulate therapy without major morbidity and mortality for limb transplantation (2,7,10,11,13,18,22,28,29,30,34,36,42,43).

We have potential techniques and knowledge in developing clinical trial of limb allo-transplantation . In experimental animal studies, the first functional animal model, the first low dose high potent immunosuppressive therapy are all developed by our laboratory. We are also studying limb graft induced GVHD , and FK-506 related nerve regeneration. With a good large animal model and experience, a human preclinical pilot study may well be performed by our group in the near future.(42)

Based on our present result, a safe and potent immunosuppression protocol is already available, and the complication of GVHD is controllable or self-limited. Before human clinical application, functional recovery under immunosuppression, chronic rejection, and moral or ethical problems of limb allo-transplantation should all be further studied.

Rat limb transplantation differs from human application in several aspects: The nerve length pending regeneration is much longer in human, the movement of human hand is sophisticated, and the large surface of human limb skin. A dog model is more similar to human limb transplantation. So a veterinary clinical dog limb transplantation is performed to study clinical problems we may encounter in clinical human limb transplantation. (4,20,21,31)

三、實驗方法

Animals:

The recipient is a seven years old female terrier suffered a right forelimb amputation below elbow of more than 3 years' duration. The recipient was in good physical condition clinically and with normal CBC, routine chemistry, and negative immitis filaria antigen test. Donor is a 2 years old male Shiba of compatible body size. The choice

of donor is based on a normal physical checkup, a normal blood CBC and chemistry screen, and negative blood RBC antigen cross match.

Before transplantation, blood sample of donor and recipient were collected and frozen in –20 freezer. Radiographic examination of the amputated limb of the recipient was taken for future reference.

Transplantation

The donor was anesthetized by intravenous pentobarbial sodium. After aseptic preparation of the surgical area, the right forelimb was removed. In brief, after a circumferencial incision around the skin above elbow joint, the muscles of upper arm including the brachiocephalicus, superficial pectoral, deltoideus, triceps, bicpes, and brachialis were incised about 2 to 3 cm away from the distal attachment to the humerus or elbow joint. The radial, musculocutaneous, ulna, and median nerves were identified marked and severed. The cephalic vein and brachial artery and vein were clamped and severed. The distal humerus was then cut with a oscillating saw. The amputated limb was put in cooled UW solution and the artery was infused with cool UW solution as well under the speed of 100 ml/hr. The donor animal was euthanatized with overdose pentobarbital sodium after the harvest of the limb.

The recipient was premedicated with atropine, acepromazine, and ketoprofen. After induction with sodium thiamylal, the animal was maintained with isoflurane and oxygen. Intravenous lactated ringer at the speed of 10mg/kg/hr was given. Respiration rate, heart rate, blood oxygen saturation, and blood pressure were monitored during anesthesia.

The right forelimb stump was removed on the recipient likewisely. The donor limb was flushed with lactated ringer's 5 mins before the reattached to the recipient. The humerus was fixated with a 4-hole bone plate. The blood vessels, and nerves, were reattached under a operative microscope with 9-0 and 10-0 nylon sutures. The muscle groups were reattached with 3-0 maxon sutures with a mattress fashion. The skin was sutured with 3-0 nylons.

The animal was given with intravenous cephazolin during the surgery. Postoperative cephalexin at the dose of 50mg/kg/day in 3 divided doses were given for 2 weeks.

Immunosuppression

Oral Fk506 and leflunomide at the dose of 0.5mg/kg/day and 5mg/kg/day respectively were given from 2 days before surgery .

Monitoring

Ultrasonic Doppler was used to confirm the patency of reanastomosed blood vessels. Blood level of FK506 was monitored as needed. Whole blood check-up was done as needed. Immunosuppressants toxicity including gastrointestinal disorder, intussusception, vomiting, and anemia were closely monitored.

Rejection was monitored clinically of graft skin erythema, swelling, and ulceration. If clinical sign of rejection was noted , a skin punch biopsy was performed and verified pathologically.

GVHD was monitored by clinical signs of recipient skin erythema and ulceration, diarrhea, and weight loss. If clinical signs of GVHD was noted, recipient blood was checked for high percentage chimerism by Y chromosome PCR and immunochemical stain of MHC class II expression on affected skin.

Functional recovery of the limb was monitored by pinch skin sensory tests and clinical observation of walking.

四、實驗結果

The amputated limb stump was significantly atrophied due to disuse. Diameter of the recipient humerus wais about 50% smaller than that of the donor. Humerus was fixed with a 4-hole bone plate at the medial side of the bone. Major incompatibility was encountered in the diameter of veins. Both recipient veins anastomosed were only one-third the diameter of the donor veins, which made the end-to-end anastomosis extremely difficult.

The whole procedure took 11.5 hours including donor harvest and transplantation. Cold ischemia time of the limb was 4.5 hours. The anastomosed vessels were patent right after the transplantation and the animal was recovered from the surgery uneventfully. The animal was with fair appetite and spirit after the transplantation.

Swelling of the transplanted limbs was noted for 15 days after the transplantation. Seroma was drained from the surgical wounds when needed and prednisolone was given at the dose of 1mg/kg/day PO for the edema. Edema was totally gone in 15 days and the dog was on routine immunosuppressive therapy. Hair was growing back since the 7th day. Antibiotics was discontinued 15 days after transplantation. FK-506 blood through level was monitored twice weekly in the first 2 weeks and once a week after that. The blood through level was maintained between 5-20 ug/ml.

The dog had one episode of severe bloody diarrhea and vomiting 25 days after transplantation. Stool floatation and routine CBC and blood chemistry were unremarkable. The gastrointestinal disorder were recovered with antibiotics (Amoxicillin 50mg/kg/day PO in 3 divided doses) and fluid therapy. In suspecting of drug toxicity, the leflunomide dosage was redued to 4 mg/kg/day since the 29 days after transplantation. One more episode of diarrhea was noted in the 31st day and readily recovered with antibiotics.

In the 40th day, very mild swelling of the transplanted limb was noted. The swelling gradually progressed into pitting edema in 4 days and the leflunomide dosage was resumed to 5 mg/kg/day and prednisolone 2mg/kg/day was given to observe the reverse of edema.

No motor function recovery of the carpal movement was noted in 50 days. However, returning of sensory function was noted on the upper part of stump skin in the 35 to 40 days.

The animal is still under close watch for signs of rejection and neural function recovery.

五、討論

In our other study, the combination of LEF and FK506 at the dose of 10mg/kg/day and 0.5mg/kg/day successfully prevented the rejection of rat limb allograft across strong histocompatibility barrier. Many of the rats even developed irresponsiveness without immunosuppression. However, because of the sensitivity to LEF toxicity of dogs, our canine study use the lefunomide dose of 5 mg/kg/day. Which, along with FK506 0.5mg/kg/day seemed to control acute rejection postoperatively quite well. The minimal reduction of the dosage cause edema to occur. The etiology of the swelling still remains unknown. However, rejection is among the most probable causes. Gastrointestinal disorder of both drugs also make very small window of these 2 drugs combination to control rejection and waive the danger of drug toxicity.

In our rat limb allotransplantation model, all limb regained sensory reaction, weight bearing, and actually walked. According to clinical , sensory reaction and walking track evaluations, the functional recovery of allografts was less favorable when compared

with our data on limb iso-transplantation. The differences may be due to immunosuppression, technical reasons, or celluar, histochemical or physiological incompatibility between different strains of rats. The functional recovery in our dog case was incomplete in 50 days, which need further observation.

In limb replantation and transplantation, postoperative edema may be due to impaired venous and lymphoid circulation, and local tissue inflammation. In dogs, neovascularization may replace the original venous circulation in 7 to 10 days after the surgery (25). In this study, the edema after limb allo-transplantation resolved in 15 days after the surgery, which maybe due to the highly incompatible diameter of veins.

In conclusion, combination LEF and FK506 at the dosage of 5mg/kg/day and 0.5mg/kg/day respectively did prevented acute rejection right after limb allotransplantation across major immunological barrier of canines However, the window of drug efficacy and toxicity is very small which need carful monitor of the patient condition. Long term drug toxicity and chronic rejection after prolonged immuno-suppression may also be a concern if life time dosing is still mandatory in clinical application of limb allotransplantation. Sensory and motor functional recovery need further evaluation. Y-Chromosome PCR will be used to detect chimerism of the patient to decide if tolerance or graft-versus-host disease can be predicted . This veterinary clinical model can be important reference for human limb allo-transplantation.

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