ORIGINAL PAPER

VITAL ORGANS TRANSPLANTATION MODELS IN RATS

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Abstract

Experimental transplantation models in rats are useful for the studies of transplantation immunology, as well as training in microsurgery. Vital organs transplantation (heart, kidney, trachea) in rats has been accepted as the most widely used model to investigate the immune mechanisms of transplantation, being easy to monitor clinically due to the rapid resumption of the function of the grafted organ. The study was performed on a group of Lewis rats (n=15), divided into 3 groups. In the first group (n=5) cervical heterotopic kidney transplantations were performed, in the second group (n=5) cervical heterotopic heart transplantations, and in the third group (n=5) orthotopic tracheal transplantations. The transplantation was performed between donor Brown Norway rats and recipient Lewis rats. Immunosuppressive therapy was instituted by administration of cyclosporine (7mg/kg), until the rats were euthanized. Mean donor operating time (mDOT) and mean recipient operating time (mROT) were measured, as well as the success rate of the transplant (GS). For all 3 types of transplantations, there was a 100% success rate, mDOT, respectively mROT varying, with the exception that only in the case of the orthotopic tracheal transplantation, mROT was significantly lower than mDOT. Useful microsurgical transplantation models were described for immunological studies which enabled the completion of complex microsurgical training, accessible for residents, as well as for medical students. The easy monitoring of the transplanted grafts and the decrease of the hemorrhaging during the procedure represent the advantages of cervical heterotopic transplantation.

Keywords*: transplant, microsurgery, heart, kidney, trachea*

Introduction

The cervical heterotopic kidney transplantations, respectively the cervical heterotopic heart transplantations, as well as the orthotopic tracheal transplantations in rats are experimental models widely used in the complex studies of transplantation immunology, rejection reaction as well as in the study of other associated pathologies [1]. The cervical heterotopic transplantation in rats is particularly used because of its shorter operating time and easy manner of objective evaluation of the graft function. The cervical heterotopic kidney transplantation was used to study the acute and, respectively, the chronic rejection reactions, as well as renal hypertension [2]. Whereas, after the heterotopic tracheal transplantation, as a result of the immune mechanisms, the rejection reaction is characterized by the complete obstruction of the airways, its orthotopic alternative predominantly associates a moderate

inflammatory infiltrate and edema of the lamina propria, due to the reepithelialization process by transfer of recipient-derived epithelium [3].

The performance of these transplants represents a real challenge for the surgeon who wishes to improve his/her microsurgical techniques, as well as for the medical student who aspires to this surgical specialty.

Materials and Methods

The study was performed in the Microsurgery Laboratory of The Clinical Emergency Hospital Floreasca of Bucharest. Lewis rats (n=15) were used, divided into 3 groups, on which the above mentioned transplantations were performed. In the first group (n=5), 5 cervical heterotopic kidney transplantations were performed, in the second group (n=5), 5 cervical heterotopic heart transplantations, and in the last group (n=5), 5 orthotopic tracheal transplantations. All the surgeries were performed in aseptic and sterile conditions, and the rats benefited from anesthesia and analgesia. The following were used: OPMI® Pico Lab (Carl Zeiss, Goettingen, Germany) operating microscope, an Aesculap microsurgical kit and 10-0 prolene suture. The mean operating times both in the donor and the recipient were measured. Immunosuppressive therapy was instituted by administration of cyclosporine (7mg/kg). The rats were euthanized after 120 days and the grafts were harvested and sent for histopathological examination.

Group 1- Cervical heterotopic kidney transplantations

Using this technique, the harvested kidney from the donor was transplanted from its anatomical position in the recipient's cervical region. The surgical plan involves the dissection of the recipient's cervical region, followed by en bloc graft resection (artery, vein, ureter), ending with the recipient' surgery.

In order to perform the graft resection, a median laparotomy followed by the exposure of the abdominal content was performed (Figure 1). The bowels were placed on sterile gauze, the renal fossa was exposed, and subsequently, the kidney with the renal pedicle and ureter was dissected (Figure 2). The vena cava and the aorta were clamped proximally to the bifurcation of the common iliac arteries to ensure an adequate blood supply to the abdominal viscera. The ureter (including the intramural segment) and its connection to the bladder were sectioned, the kidney was freed from the retroperitoneal space, the renal artery and the renal vein were identified and sectioned free from the surrounding tissues and severed. The graft was preserved in a 10 ml (4˚C) Ringer solution containing heparin.

Figure 1 - Median laparotomy

Figure 2 - Renal graft

A 2 cm midline incision in the cervical region was performed on the recipient, from the submaxillary region to the sternal notch. The subcutaneous tissue was dissected to create a pocket for the graft. The common carotid artery and, respectively, the external jugular vein were dissected free from the surrounding tissue (Figure 3). The actual transplant consists from end-to-end anastomoses between the renal artery and the common carotid artery (Figure 4), respectively the renal vein and the external jugular vein (Figure 5). A cutaneous

ureterostomy was performed and the ureteral lumen was maintained open by means of catheterization. The catheter was secured and a skin suture was oversewn with 4-0 prolene thread (Figure 6). The subsequent histopathological assessment of the transplant revealed congestive glomeruli and mild hydronephrosis.

Group 2- Cervical heterotopic heart transplantations

The dissection of the anterior cervical region was similar to group 1, with the exposure of the common carotid artery and of the external jugular vein. In the donor, the mediastinum was surgically exposed, the superior vena cava and the inferior vena cava were ligated, the pulmonary artery and the aorta were dissected freed from the surrounding tissues and severed (Figure 7). The vessels were ligated posteriorly to the right atrium (Figure 8). The lumen of the donor's thoracic aorta was reduced by 50% - 75% with 8.0 suture (Figure 9). On the donor's thoracic aorta an end-to-end oblique anastomosis was performed with the recipient's common carotid artery with a 10-0 suture. A subcutaneous pocket was created in order to accommodate the transplanted heart, and in the end a skin suture was oversewn with 4-0 prolene thread. The heart resumed its function with a sinus rhythm after 2 minutes.

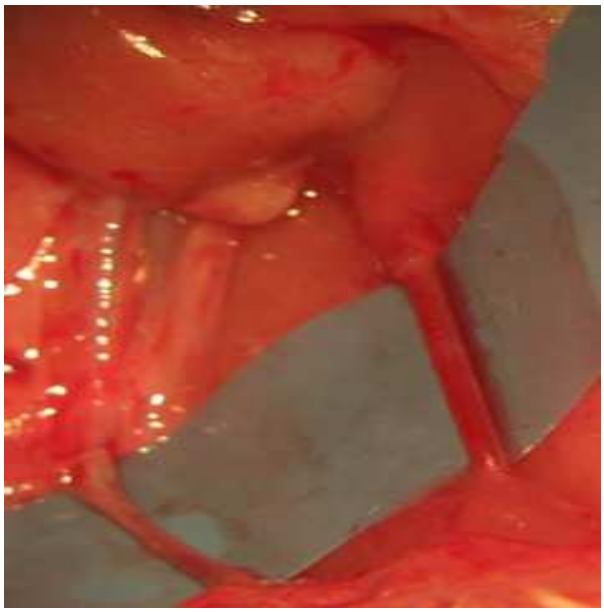

Figure 3 - Exposed common carotid artery and external jugular vein

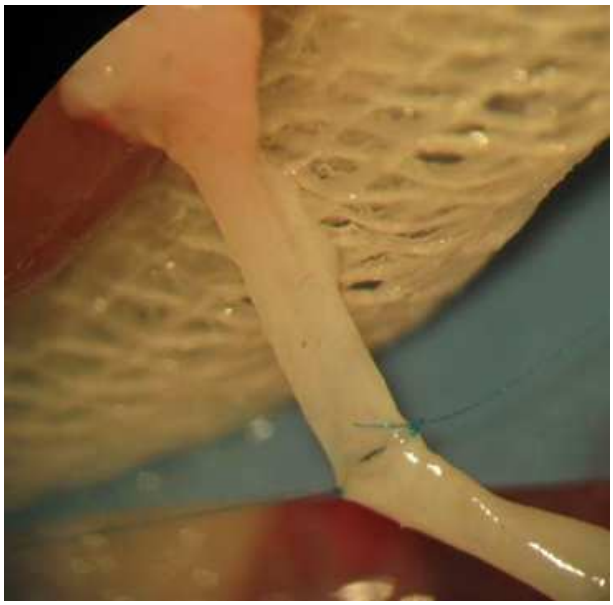

Figure 4 - End-to-end anastomosis between the renal artery and the common carotid artery

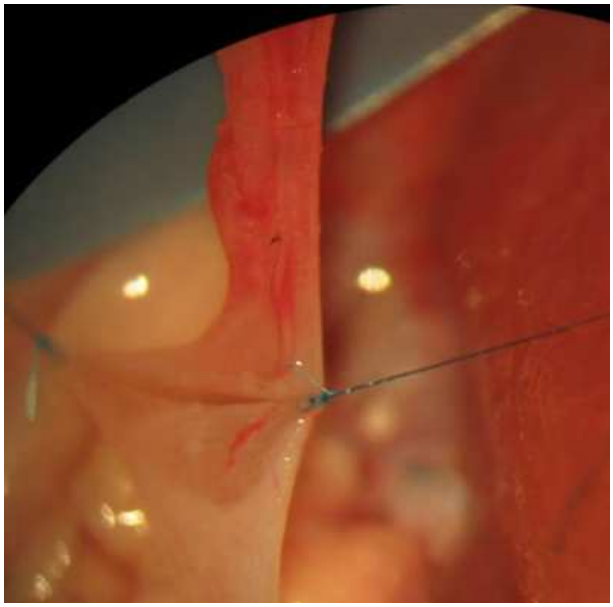

Figure 5 - End-to-end anastomosis between the renal vein and the external jugular vein

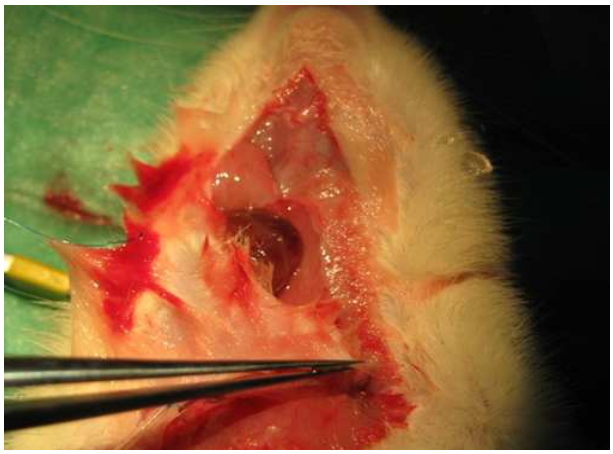

Figure 6 - Cutaneous ureterostomy

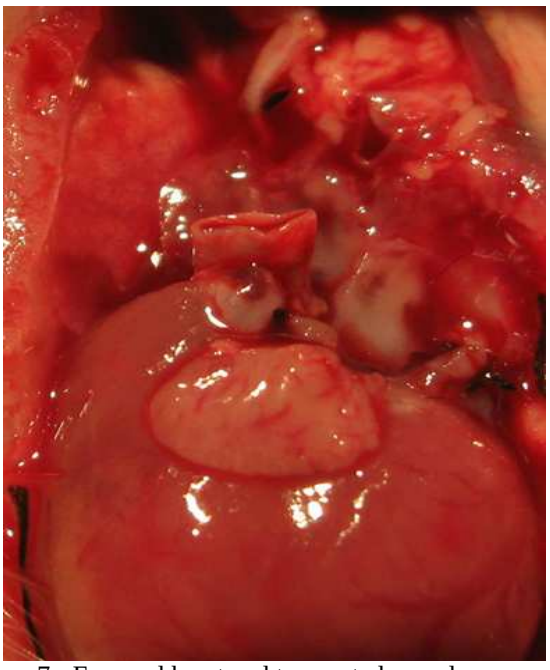
Figure 7 - Exposed heart and transected vessels

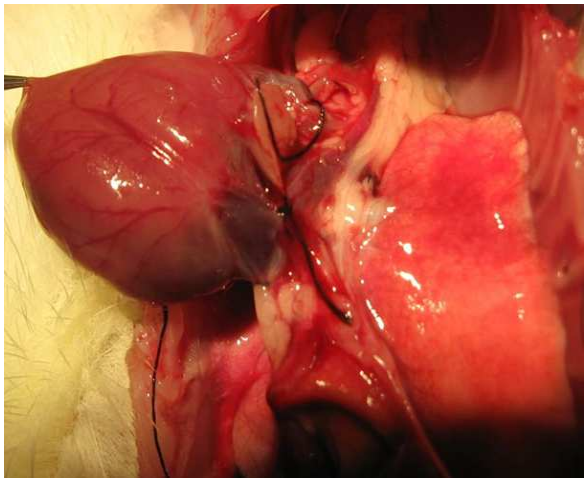
Figure 8 - Ligature of the vessels posteriorly to the right atrium

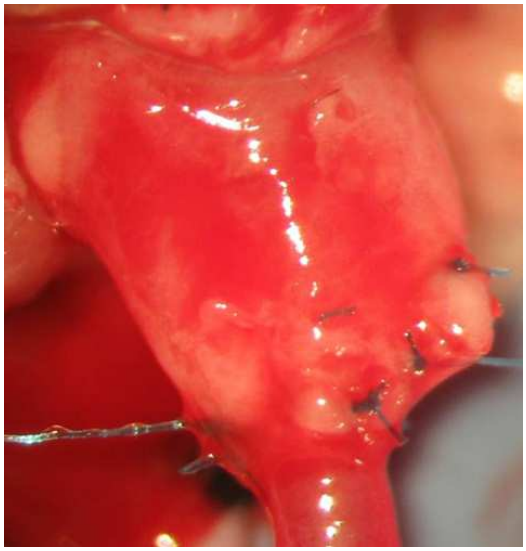
Figure 9 - Decrease in diameter of the thoracic aorta

Group 3- Orthotopic tracheal transplantations

An anterior midline longitudinal incision was made in the donor's cervical region. The division of the adjacent muscular fasciculi enabled the identification of the entire laryngotracheal complex (Figure 10). The cervical esophagus and the associated vascular structures were dissected with the preservation of the integrity of the recurrent laryngeal nerve. A circumferential tracheal segment made up of 5 rings was excised. A tracheal segment made up also of 5 rings was excised from the recipient, and was replaced with the segment harvested from the donor. The tracheal graft was secured with 10-0 interrupted transtracheal sutures (Figure 11). The sectioned muscular strata and the skin were sutured (Figure 12). Postoperatively, the respiratory frequency and the amplitude of the thoracic movements were evaluated constantly. Subsequently, obliterating tracheitis was histopathologically noticed.


Figure 10 - Laryngotracheal complex

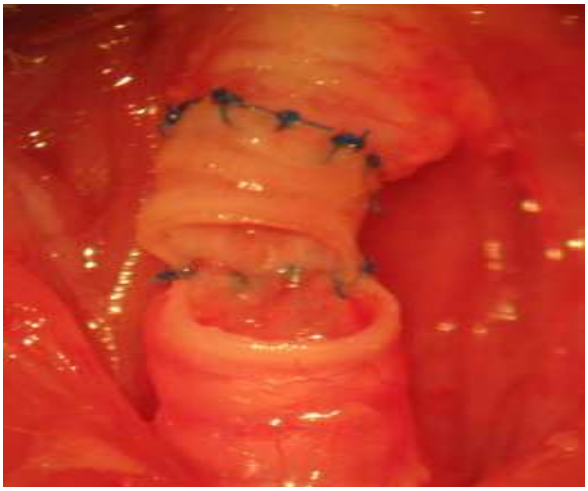
Figure 11 - Transtracheal suture

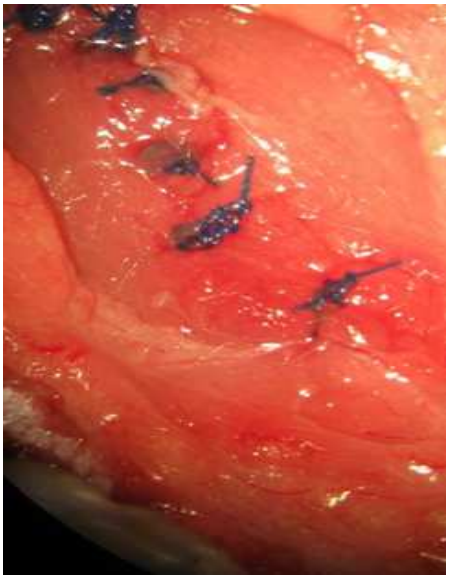

Figure 12 - Suture of the muscle planes

Results

The transplanted grafts from the experiment had a 100% success rate (GS). The mean operating times both in the donor and the recipient were measured. The shortest mean donor operating time (mDOT) was obtained in the orthotopic tracheal transplantation (35 minutes). In the case of the cervical heterotopic heart transplantation as well as the cervical heterotopic kidney transplantations, the mean operating time was longer (55 minutes). The mean recipient operating time (mROT) was approximately equivalent in the first two groups, with the specification that, in the third group, the mROT was of 20 minutes. The revascularization time in the second group was of 2 minutes. Histopathologically, in the third group, an obliterating disease was noticed, and in the first group mild hydronephrosis with congested glomeruli.

	Group 1	Group 2	Group 3
mDOT	55 minutes	55 minutes	35 minutes
mROT	60 minutes	70 minutes	20 minutes
GS	100 %	100 %	100 %

Table 1 - Results

Disscusions

The reproduction of the transplantations described in this article requires vascular dissection techniques, in order not to compromise the integrity of the graft harvested from the donor. Likewise, during the process, the ischemia time must be less than 30 minutes, in order not to additionaly interfere with the physiopathologic processes taking place postoperatively. The carefull mobilization of the graft, without subjecting it to additional mechanical shocks, besides maintaining the vascular patency, are absolutely essential in order to obtain a high survival rate.

An essential stage in the cervical heterotopic kidney transplantation is the execution of the cutaneous ureterostomy. The surgical difficulty derives both from the extremely small ureteral diamenter, and from its excessive fragility [5]. The complications arising in the case of the abdominal kidney transplantation have been successfully avoided: the abdominal amassment of urine, with acute peritonitis and necrosis. Postoperatively, complications such as urinary complications, massive hemorrhages, arterial anastomotic thrombosis or air embolism, as reported by Liang Zhu et al [1], were not encountered

The cervical heterotopic heart transplantation is preferentially used, in comparison to its abdominal alternative, on account of multiple considerations, among which the following should be mentioned: less surgical difficulty, significantly reduced hemorrhage, shorter operating time, capacity of objective examination of the graft by palpation (in case of rejection, the force of the myocardial contraction decreases considerably up to the point when it becomes unnoticeable), electrocardiogram, Doppler echography. The surgical difficulty is amplified by the increased risk of thrombosis, the minimal thickness of the venous wall, as well as by the significant difference in diameter between the donor's vessels and the recipient's vessels. Thus, vascular reconstruction was performed by reducing the diameter of the donor's thoracic aorta by 50%-75%, prior to the end-to-end anastomosis with the common carotid artery [4].

Ischemia, infection and increase of the fibroblastic activity have been quoted by Eric M. Genden et al as being responsible for the occurrence of obliterating tracheitis in the case of heterotopic tracheal transplantation. This pathology that does not occur so aggressively in the case of orthotopic transplantation because of the reepithelialization process with recipient-derived epithelium [3]. Moreover, it is essential during the transplantation to maintain the graft polarity, in order not to disturb the mechanism and operating principle of the ciliated respiratory epithelium. In our experiment, we admit that obliterating tracheitis with airway edema and inspiratory stridor occurs.

Conclusions

- Microsurgical models feasible for transplantation have been described, which are useful for the studies of transplant immunology as well as complex microsurgical training. In order to perform the above mentioned transplantations, basic vascular microsurgical techniques were applied in a classic microsurgery laboratory. These experimental transplantation models can be achieved both by the plastic surgery and reconstructive microsurgery resident and by the medical student who wishes to have a complex microsurgical training which involves the blending of general knowledge of animal anatomy, precision and patience in performing the microsurgical techniques.

References

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