

Video Article

Non-invasive Imaging of Acute Allograft Rejection after Rat Renal Transplantation Using ¹⁸F-FDG PET

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Abstract

The number of patients with end-stage renal disease, and the number of kidney allograft recipients continuously increases. Episodes of acute cellular allograft rejection (AR) are a negative prognostic factor for long-term allograft survival, and its timely diagnosis is crucial for allograft function¹. At present, AR can only be definitely diagnosed by core-needle biopsy, which, as an invasive method, bares significant risk of graft injury or even loss. Moreover, biopsies are not feasible in patients taking anticoagulant drugs and the limited sampling site of this technique may result in false negative results if the AR is focal or patchy. As a consequence, this gave rise to an ongoing search for new AR detection methods, which often has to be done in animals including the use of various transplantation models.

Since the early 60s rat renal transplantation is a well-established experimental method for the examination and analysis of AR². We herein present in addition small animal positron emission tomography (PET) using ¹⁸F-fluorodeoxyglucose (FDG) to assess AR in an allogeneic uninephrectomized rat renal transplantation model and propose graft FDG-PET imaging as a new option for a non-invasive, specific and early diagnosis of AR also for the human situation³. Further, this method can be applied for follow-up to improve monitoring of transplant rejection⁴.

Video Link

The video component of this article can be found at <http://www.jove.com/video/4240/>

Protocol

1. Donor Organ Recovery

1. Set up the stereotactic microscope, record weight of the 8-10 week old rat (donor and recipient body weight should match).
2. Anesthetize the donor rat (Lewis Brown Norway F1, LBN F1) using oxygen/isoflurane inhalation (isoflurane 4 %/2 L/min oxygen). Maintain anesthesia by lowering isoflurane to 2-2.5%.
3. Place the anesthetized rat on the surgery pad. Fix the rat's extremities with adhesive tape onto the pad and apply ophthalmic ointment (Bepanthen, Bayer) to the rat's eyes.
4. Shave and disinfect the abdomen of the rat and perform a ventral midline incision from the pubis to the caudal border of the liver. Expose the left kidney and its vessels by carefully moving the intestine to the right side. Place a thin sheet of gauze over the left kidney and moisten it with warmed isotonic saline solution to prevent desiccation.
5. Carefully dissect the fat tissue with Dumont Angled Tip Forceps (Dumont SS-45 Forceps Inox Medical, Fine Science Tools, cat.no. 11203-25) enveloping the left ureter. Avoid any direct contact with the ureter, instead, mobilize it with sufficient surrounding fat tissue.
6. Separate the left renal vein from the renal artery using Dumont Angled Tip forceps. Remove all fat and connective tissue from both vessels and cauterize the adrenal and testicular vessels.
7. Dissect aorta and inferior vena cava (IVC) above and below its junction with the left renal artery and vein, respectively, and cauterize all outgoing arteries.

8. Clamp the suprarenal aorta with a microsurgical clamp (Micro Serrefines, Fine Science Tools, cat.no. 18055-04) above the left renal artery, as near as possible to the superior mesenteric artery. Ligate the infrarenal aorta using surgical silk (5-0, Vömel, art no. 14739) approximately 5 mm below the left renal artery.
9. Ligate the infrarenal IVC with surgical silk (5-0, Vömel), and clamp the suprarenal IVC with a microsurgical clamp (Micro Serrefines, Fine Science Tools, cat.no. 18055-03).
10. Cut the renal vein as close as possible to the IVC using fine scissors (Iris Scissors - ToughCut Straight 11.5 cm, Fine Science Tools, cat.no. 14058-11).
11. Slowly perfuse the kidney *in situ* with 2 ml ice-cold HTK perfusion solution (CUSTODIOL HTK, Dr. Franz Köhler Chemie) by inserting a canula (Microlance 3 27G $\frac{1}{4}$, BD, cat.no. 302200) in the infrarenal aorta. Confirm that the renal color changes (now olive-tinted).
12. First, transect the suprarenal aorta, then the infrarenal aorta and finally the ureter as close as possible to the urinary bladder.
13. Remove the kidney and its vascular supply together with the ureter and store in HTK perfusion solution on ice.
14. Euthanize the donor rat by removing the vascular clamps and consecutive excision of the heart under anesthesia.

2. Recipient Preparation and Transplantation

1. Set up the stereotactic microscope, record weight of the rat.
2. Anesthetize the recipient rat (Lewis) using isoflurane 4%. Maintain anesthesia by lowering isoflurane to 2-2.5%.
3. Place the anesthetized rat on the surgery pad. Fix the rat's extremities with adhesive tape onto the pad and apply ophthalmic ointment to the rat's eyes. Monitor and control core body temperature using a rectal temperature sensor and a warming pad. During surgery repetitively control temperature, breathing and heart rate of the animal.
4. Shave and disinfect the abdomen and perform a ventral midline incision from the pubis to the caudal border of the liver. Use a scalpel forcutting the skin in order to minimize skin trauma. Expose the left kidney and its vessels by moving the intestine to the right side.
5. Carefully remove the renal capsule using Dumont Angled Tip forceps and cotton swabs.
6. Occlude the left renal artery, vein and ureter near the renal hilum with two ligations using surgical thread (Mersilene 4-0, Ethicon, cat. no. EH6732H). Excise the greater part of the kidney, leaving only the renal hilum. Clean the cut surface with cotton swabs and check for bleeding. Add another ligature if necessary.
7. Carefully dissect bluntly through the connective tissue covering the infrarenal aorta and vena cava on the caudal site with cotton swabs.
8. Separate any visible nerve from the infrarenal aorta and vena cava using Dumont Angled Tip forceps.
9. Carefully dissect through the connective tissue sheet between the infrarenal aorta and vena cava, forming two openings of 2-4 mm length: The first below the branching of the testicular vessels, and the second one above the branching of the common iliac vessels. Cauterize the iliolumbar arteries and veins in between.
10. Draw a single surgical thread (Mersilene 0; Ethicon, cat. no. EH6665E) through each of the two openings. Using these threads pull the infrarenal aorta and vena cava gently to the right side of the animal to gain access to the vessels branching on the dorsal site. Cauterize any of these vessels between the two openings and remove the surgical threads thereafter.
11. Next, occlude aorta and IVC by clockwise application of four microsurgical clamps (Micro Serrefines, Fine Science Tools, cat.no. 18055-04) beginning at the upper opening with the aorta and ending with the vena cava at the upper opening (Micro Serrefines, Fine Science Tools, cat.no. 18055-03).
12. Afterwards carefully pierce the upper ending of the isolated part of the aorta with a needle (Microlance 3 27G $\frac{1}{4}$, BD, cat.no. 302200), insert the lower blade of a fine spring scissor (Vannas Spring Scissors - 3 mm Blades, Fine Science Tools, cat.no. 15000-00) through the puncture opening and perform a short longitudinal incision. Flush the isolated aorta with ice-cold HTK perfusion solution to remove any remaining blood.
13. Likewise, carefully pierce the lower ending of the isolated part of the vena cava with a needle, insert the lower blade of a fine spring scissor through the puncture opening and perform a longitudinal incision ending shortly below the aortic incision. Again, flush the vessel to remove any remaining blood.
14. Remove the kidney graft from the storage solution. Place it below the former position of the left kidney, and ensure that it is correctly oriented.
15. Check the orientation of the grafts renal artery and ensure that there are no twists. Then place a thin sheet of gauze over the graft and moisten it with ice-cold HTK perfusion solution to cool it, and to prevent desiccation.
16. Fixate the opening of the suprarenal part of the aortic piece in which the renal artery of the graft opens into with surgical thread (Ethilon 9-0, Ethicon, cat. no. 2809G) - first at the upper, then at the lower end of the aortic incision. Close the incision clockwise with a continuous suture using surgical thread (Ethilon 9-0), Dumont Angled Tip forceps and a curved Castroviejo needle holder (Fine Science Tools, cat. no. 12061-01). Afterwards, cover the first suture with connective tissue through a counterclockwise second continuous suture. Now, only the lower part of the aortic piece of the graft remains to be closed. Regularly cool the graft with ice-cold HTK perfusion solution.
17. Perfuse the graft with 1 ml of ice-cold HTK perfusion solution through a blunt needle inserted into the open end of the grafts aortic piece validating the tightness and integrity of the suture, as well as removing remaining blood in the graft. Afterwards, ligate the open end of the aortic piece with surgical silk (5-0, Vömel).
18. Check the orientation of the grafts renal vein and ensure that there are no twists.
19. Fixate the opening of the vein of the graft with surgical thread (Ethilon 9-0) - first at the upper, then at the lower end of the incision at the vena cava. Close the incision clockwise with a continuous suture using surgical thread (Ethilon 9-0), Dumont Angled Tip forceps and a curved Castroviejo needle holder. Regularly cool the graft with ice-cold HTK perfusion solution.
20. Counterclockwise remove the microsurgical clamps, beginning with the uppermost vena cava clamp. After removing the last aortic clamp, carefully stop eventually mild bleeding with cotton swabs. After a few seconds the graft will turn rosy-tinted, and uretric contractions should appear.
21. For the insertion of the grafts ureter into the recipient's bladder carefully remove a small patch of the musculature at the top of the bladder using a Vannas Spring Scissor (Student Vannas Spring Scissor straight, Fine Science Tools, cat.no. 91500-09). Then, remove the surrounding tissue (mostly fat) from the graft ureter using Dumont Angled Tip forceps. Therefore, carefully grab the tip of the ureter and gently separate the surrounding fat, connective tissue and the embedded vessels from it by pulling them in the opposite direction. Fixate the uretric tip at the end of a surgical thread (Prolene 6-0, Ethicon, cat. no. 8697H) and perforate the previously prepared top of the bladder with the attached needle. Pull the ureter through the bladder and exit it at the base through a second perforation.

22. Fixate the previously from the ureter separated tissue at the top of the bladder with surgical thread (Ethilon 9-0). Cut off the tip of the ureter with the attached surgical thread and pull the ureter back into the bladder by gently pushing from below. Afterwards, close the second bladder puncture with surgical thread (Ethilon 9-0).
23. Close the abdominal wall and skin by two continuous but separate sutures using surgical suture thread (Mersilene 0). Disinfect the wound using Povidon Iodine and administer buprenorphine (0.1 mg per kg/BW/bid) s.c. for three days after surgery to control wound pain.

3. Imaging Allograft Rejection

1. Anesthetize nonfasting rat using isoflurane 2-2.5%.
2. 30 MBq FDG (30 MBq FDG in 0.1 ml 0.9% NaCl) are administered i.v. via catheterizing a lateral tail vein using a 24 G catheter (Braun, Introcath, 4252500-01). Afterwards, purge the catheter with 0.9 ml 0.9% NaCl solution.
3. Leave the rat in a restrainer without anesthesia until start of the scan and hydrate the animal by i.v. injection of 1 ml of 0.9% NaCl solution hourly.
4. Re-anesthetize rat using isoflurane 2% immediately before the scan starts.
5. Place anesthetized rat in a high-resolution multi-wire chamber-based animal PET camera quadHIDAC (Oxford Positron Systems Ltd, Oxford, UK). The spatial resolution of the PET scanner is 1.0 mm and is constant over the whole FOV (diameter, 165 mm; axial length, 280 mm). Monitor and control body temperature during the scan using a rectal temperature sensor and control anesthesia with a pulse oximeter.
6. Start dynamic acquisition for 60 min starting 180 min after FDG-injection to reduce tracer accumulation in the kidneys caused by renal excretion of FDG.
7. Apply 5 MBq of ^{18}F -fluoride i.v. and perform another PET scan immediately after ^{18}F -fluoride injection for 60 min without moving the position of the rat in the scanner for identification of renal parenchyma and for calculation of ^{18}F -clearance⁵. Reconstruct images from both scans. Trace manually a volume of interest (VOI) on reconstructed images 2 min after ^{18}F -fluoride injection (perfusion-phase) around renal cortex and transfer the VOI to the FDG images. Carefully exclude the renal pelvis from the VOI. Reconstruct images from both scans and manually trace a volume of interest (VOI) around renal cortex. List-mode data were reconstructed into images with a voxel size of $0.4 \times 0.4 \times 0.4 \text{ mm}^3$. Carefully exclude the renal pelvis from the VOI.
8. Compute FDG uptake by the ratio of total counts and volume and calculate the percentage of injected dose (%ID). We used MATLAB (Version R2011b, MathWorks, Natick, MA, USA) and tomographic imaging (TIM) Version 2.8 for image analysis.

Representative Results

Histology

During AR leukocytes, *i.e.* mainly T-lymphocytes are recruited into the transplant, whereas the severity of the rejection is reflected by the degree of inflammation. In the Periodic-Acid-Schiff (PAS) staining depicted here (**Figure 1**), the renal allograft shows significant histological signs of AR, namely glomerulitis, tubulitis, endothelialitis and graft infiltration (**Figure 1**, aTX POD4) (POD = postoperative day) while signs of rejection are absent in the native control kidney (**Figure 1**, CTR). Graft infiltrating cells are highly metabolically active cells which consume large amounts of glucose. However, if the latter is substituted with FDG, this will accumulate in the cells and can be measured and quantified by PET.

PET Images

Representative PET images of dynamic whole body acquisitions of a series of an allogeneically transplanted rat after tail vein injection of 30 MBq FDG (maximum a posterior MAP projection, 180 min p.i.) (**Figure 2**). A typical FDG distribution is found with distinct physiological accumulation in brain, heart, bone marrow and harderian glands. Moreover, free filtrated FDG accumulates in the urinary tract. In renal grafts undergoing AR the parenchyma (yellow circle, left kidney) highly accumulates FDG with a maximum on POD4, whereas the native kidney (green circle, right kidney) does not show any accumulation at all. Since the renal pelvis can contain free eliminated FDG, it was excluded from further measurements. Figure taken from³.

Quantitative evaluation

For quantitative evaluation images were reconstructed, volumes of interest were traced manually around the kidneys according to ^{18}F -fluoride perfusion and projected on the FDG images. After exclusion of the renal pelvis mean FDG uptake in the renal parenchyma was calculated by the ratio of total counts to volume (%ID \pm SEM). Kidneys developing AR showed significantly increased FDG accumulation on POD4 ($0.8 \pm 0.06\%$) when compared to native controls ($0.2 \pm 0.02\%$) or syngeneically transplanted kidneys ($0.37 \pm 0.04\%$). Moreover, two major differential diagnoses of AR, namely acute tubule necrosis like in ischemia/reperfusion injury (IRI) ($0.31 \pm 0.02\%$) and acute calcineurin inhibitor toxicity (CSA) ($0.16 \pm 0.01\%$) did not show an elevated FDG accumulation and can therefore be distinguished from AR (**Figure 3** taken from³).

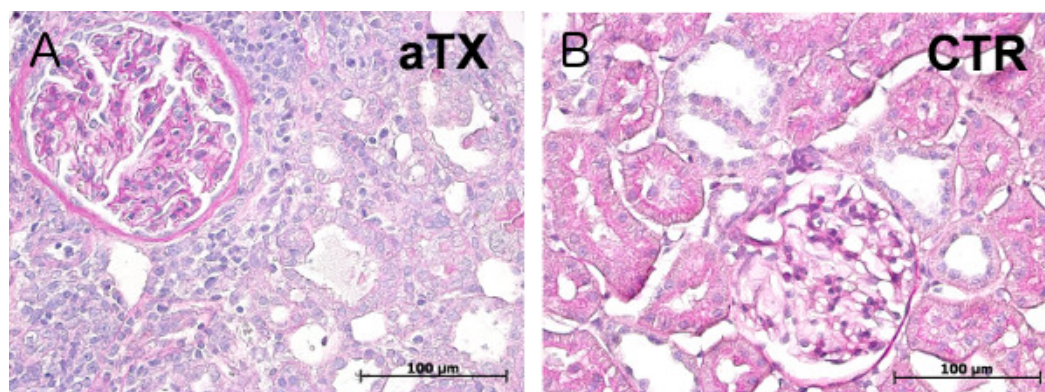


Figure 1. Histology. Signs of acute rejection, namely glomerulitis, tubulitis, endothelialitis, and graft infiltration, were found in the allograft group (aTX) and were completely absent in control kidneys (CTR).

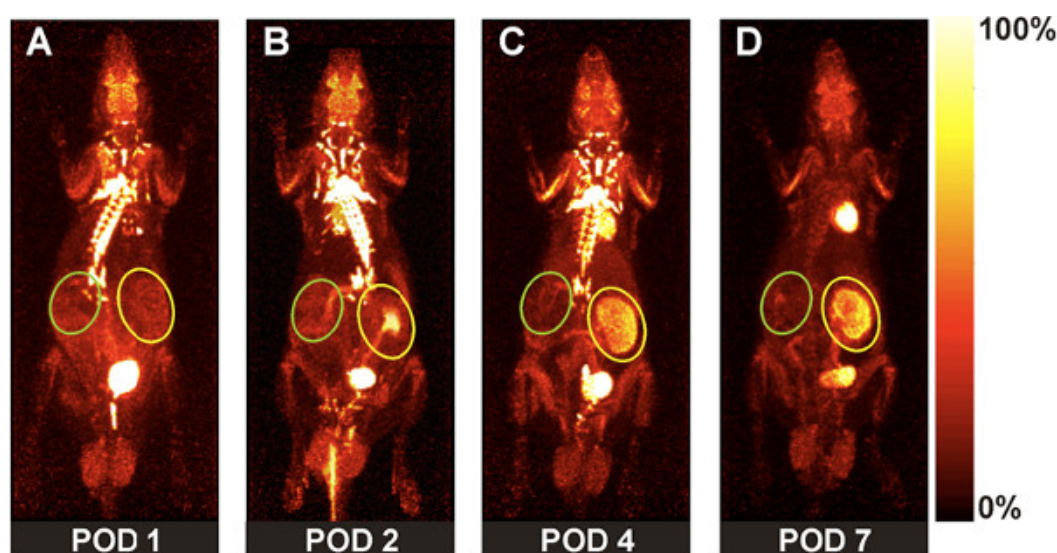


Figure 2. FDG-PET Image. Representative PET-images of dynamic whole body acquisitions of a series of an allogeneically transplanted rat. In comparison to control kidneys (green circles) accumulates the parenchyma of renal allografts (yellow circles) FDG with a maximum on POD4. Since the renal pelvis can contain free eliminated FDG it was excluded from the measurements. Figure taken from ³.

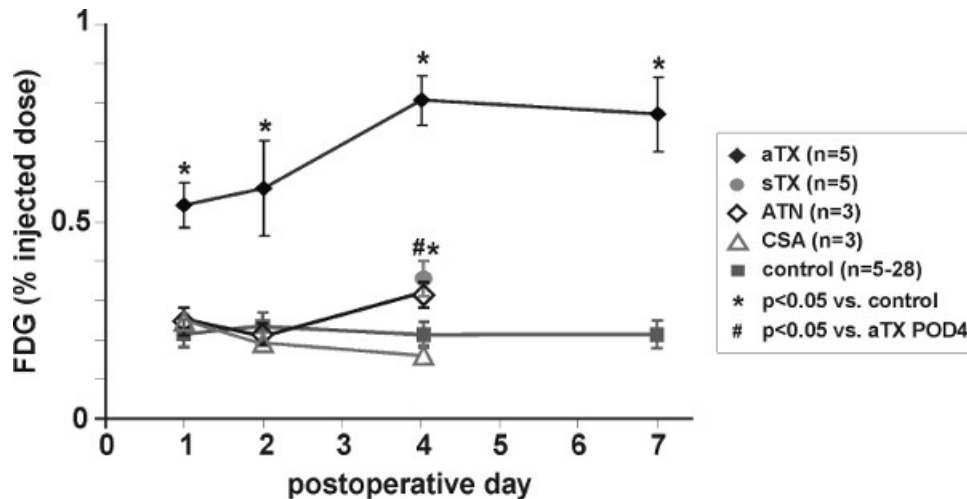


Figure 3. Quantitative evaluation. Detection of acute rejection by measurement of the %ID of FDG. Renal allografts (aTX) exhibit significant higher FDG accumulations than control kidneys (CTR), syngeneically allografts (sTX), kidneys with acute tubule necrosis (ATN) or kidneys with acute calcineurin inhibitor toxicity (CSA) with a maximum on POD4 (aTX: $0.8 \pm 0.06\%$ CTR: $0.2 \pm 0.02\%$, sTX: $0.37 \pm 0.04\%$, ATN: $0.31 \pm 0.02\%$, CSA: $0.16 \pm 0.01\%$). Figure taken from ³.

Discussion

FDG-PET imaging is a new option for the diagnosis of acute rejection. Because of its non-invasive and specific nature, FDG-PET is advantages in comparison to classical diagnostics by core needle biopsy. In contrast to the limited sample size of a biopsy, FDG-PET analysis the whole graft. Moreover, one can apply it to patients on anticoagulant therapy, and one can perform PET measures repetitively e.g. to monitor treatment efficiency ⁴. In addition, we already have shown that two major differential diagnosis of AR, namely acute tubule necrosis caused by ischemia reperfusion injury and acute calcineurin inhibitor toxicity, can be differentiated from AR using FDG-PET ³. Since FDG-PET imaging requires relatively low amounts of activity and imaging of either transplant patients or/and patients with impaired renal function is not associated with an increased risk of severe complications this approach can be easily transferred into daily clinical routine.

Nevertheless, one has to keep in mind that FDG is a rather non-specific tracer assessing regional metabolic activity. Thus, graft infection or tumors might lead to false positive results as well. Drainage of FDG into the renal pelvis might be a problem when assessing FDG uptake in the renal parenchyma. Therefore, we have chosen a late acquisition time three hours after injection to reduce tracer accumulation in the kidneys caused by renal excretion of FDG. In addition, the renal pelvis has to be carefully excluded when quantifying the renal FDG uptake. According to this protocol PET can be used to non-invasively detect AR, to differentiate it from ATN and CSA, and to perform serially investigations for follow-up or for evaluation of treatment efficiency ^{3,4,6}. PET using ¹⁸F-fluoride is also useful to assess (split) renal function by calculation of the renal fluoride clearance as published before ⁵.

The allogeneic renal transplantation model using LBN F1 donor and Lewis recipient rats is an ideal model for investigation of acute cellular allograft rejection. In the absence of immunosuppression the allograft kidneys develop typical histological signs of AR according to the BANFF classification ⁷. Further, depending on the chosen modality (uni- vs. binephrectomized transplantation ^{3,8-10}) metabolic data can be evaluated as well to monitor allograft function. Due to the absence of immunosuppressive treatment, wound or systemic infections of the rats are extremely rare. Common surgical complications of this model include vessel stenosis, mostly seen at the insertion points of the graft vessels into the aorta or IVC of the recipient. This might cause ischemia or graft thrombosis. One can avoid this complication by using longer incisions in aorta and IVC. Sometimes uremia is found due to graft failure or ureter leakage caused by ureter necrosis or disconnection of the ureter from the bladder. If signs of uremia e.g. apathy, loss of appetite or spontaneous weight gain occur, the animals should be euthanized immediately.

Disclosures

No conflicts of interest declared.

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