A new murine model of endovascular aortic aneurysm repair

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Introduction

Histological and biochemical modifications after aneurysm endograft exclusion are misunderstood. We describe a new model of endograft implantation on a murine aneurysm. Thrombus analysis, endoleaks consequences and materials used for endovascular repair could be tested before human implantation.

Methods

Aortic abdominal aneurysms (AAA) is induced by elastase perfusion in Wistar rats, aged from 8 to 9 weeks as previously described by Anidjar. [1]

Two to 4 weeks later, the rat is re-operate, and a coronary covered stentgraft of 3mm of diameter is used to exclude the aneurysm. The graft is deployed under direct vision control, after insertion into the distal aorta throught an aortotomy.

Two weeks later, an aortography from the left common carotid artery is performed to confirm the stentgraft patency. The rat could then be sacrified and the stented aorta, including the stentgraft, the thrombus and the aortic wall is harvested for analysis.

Protocol

Step 1 : Abdominal aortic aneurysm induction

Material:

Animals : Wistar rats, aged from 8 to 9 weeks. Weight range from 350 to 400g.

Instruments :

- Isoflurane anesthesic system : 4,5% at the beginning, then 2%.
- Penthobarbital
- Dissection stereomicroscope
- Steriles gloves
- Microsurgical Steriles instruments :
    - Needle holder
    - Forceps
    - Scissors
    - Gilbert approximator
- N°40 Silk string
- Prolen 9-0 and 10-0
- Heat-tapered polyethylene tubing (PE 10)
- Syringe infusion pump
- Porcine pancreatic elastase (Sigma, St. Louis, Mo., USA)
    - 550µl of salin is mixed with 175µl of elastase
- 3 Micro clamps
- Inflator
- 9 to 16mm length, 3mm diameter coronary covered stentgraft

Day 0 :

After a short anesthesia with isoflurane, an intra peritoneal penthobarbital injection is performed. With 0,1ml for 100g dilued in 0,1ml of salin serum for 100g, anesthesia is long enough for the procedure.

After shaving the abdomen, the skin is cleaned with alcohol.

A xypho pubic laparotomy is performed. Spreaders are placed and all the intestins are wrapped into a wet compress on the left side of the animal.

The peritoneum is opened directly above the aorta, without tearing it. The goal is to prevent structures adhesions at the re-operation time with a very economic dissection.

All the collaterals from the left renal vein to the iliac bifurcation are ligated with only one ligature at the origin of the aorta.

If the aorta is stuck to the bowels or the vena cava, there‘s a high risk of bleeding during the second intervention, which could be often fatal for the rat.

It‘s important to dissect the aorta entirely near the left renal vein and just before the iliac bifurcation to insert the Gilbert approximator clamps, and approximately 15mm below the left renal vein. A 40 silk is turned around the aorta two times, 15mm below the left renal vein.

After clamping the aorta, the adventice is removed, and a little aortotomy is performed. Aorta has to be cleaned very carefully to wash all the remaining blood. Otherwise, clots could migrate when declamping.

Heat-tapered polyethylene tubing (PE 10) is smoothly introduced inside the aorta, and secured with a tie to avoid pressure expuslion.

Injection is made into a 10 to 13mm lenght of aorta (from the proximal clamp to the silk).

We inject 725 µl of solution at constant speed during about 40 min. It is important to carefully survey the injection : the aorta should expense from 140-150% of its normal diameter.

At the end of the injection, the catheter is removed, the silk is cut and the aortotomy is closed with about 3 stitches of 10-0 Prolen.

Abdominal wall is closed with a continuous absorbable suture, and the skin with discontinued non absorbable sutures. A 5mg/Kg dose of Carprofen is injected subcutaneously to prevent pain.

The rats are then returned to their cage and given the standard rat laboratory chow and water *ad libidum*.

<u>Step 2 : abdominal aortic aneurysm exclusion with a coronarian covered stentgraft.</u>

Day 15-30 :

After anesthesia as previously described, redux laparotomy is performed, watching carefully of the potential internal organs stucked to the abdominal wall.

Aorta is free above the aortic bifurcation, and at the proximal neck.

Aneurysm should be a few millimeters below the left renal vein, which allows a satisfactory length between the distal neck and the distal clamp to insert the catheter, and suture the aorta.

Stentgraft length depended on the aneurysm size, in order to cover both proximal and distal necks of around 2 millimeters.

A double loop silk is wrapped around the proximal neck. Aorta is clamped above the aneurysm and below the left renal artery, and above the aortic bifurcation.

A large aortotomy to avoid aorta tearing wall when introducting the stentgraft is performed. The aorta is not rinced to preserve integrity of the thrombus, and the stengraft is pushed towards the proximal clamp. Clamp is removed. Controlled tension of the silkj allow shaft progression.

Under direct sight control, stentgraft is deployed using an inflator syringe at a 8 atm pression. Balloon is then deflated while the silk is tighten again, then the shaft is removed.

The stentgraft is washed with saline serum, a clamp is placed just below the distal neck, and aorta is stitched with few sutures of 10-0 Prolen.

Wound is closed, Carprofen isinjected subcutaneously and the animals return to their cage and are given the standard laboratory chow and water *ad libidum*.

Step 3 : control of stentgraft patency.

15 days after stentgraft insertion, an arteriography is performed to control graft patency.

After anesthesia, left common carotid artery is exposed, its distal part is ligated and a 18G catheter is inserted retrogradely. Contrast is injected throught the catheter under direct fluoroscopy.

The rat are then sacrified, and the aneurysm, stentgraft, thrombus and aortic wall are harvested and sent for histological or biochemical studies.

Discussion

Two models of AAA have been already described in our lab : xenograft model [2] and elastase induced.[1] We prefer to use the elastase model because it seems to be the most similar to human aortic aneurysms.

Elastase induced AAA in rats present a large intraluminal thrombus all around the aneurysm, which is excluded by endovascular aneurysm repair.

Endoleaks are an usual complication of Endovascular Abdominal aortic aneurysm repair (EVAR)[3, 4].Lots of questions remain unsolved : AAA rupture have been described with low endoleaks, and sometimes without aneurysm sac enlargement[5]. Type 1 endoleaks are a very usual complication after EVAR, maintaining an interface between blood circulating cells and the thrombus.

As long as the aneurismal bag is perfused, proteoytic activity secondary to clot formation, neutrophils, bacterias[6, 7]…carry on.

Type V endoleaks, also named endotension, cannot only explain aneurysm growth by themselves[8]. Enzymatic activity could be part of this unsolved problem.

Actually, only covered stentgrafts have been deployed. Many ways of research are planned to be explored : implanting non covered endografts, testing the flow divertion materials, enriching the thombus activity by porphyromonas gingivalis injections[9]...

This model of endovascular exclusion of an aortic aneurysm is the first described in the litterature in the small animal. We believe that this is a start for a better understanding of excluded aneurysm remodeling[10]. Aneurysm stentgraft exclusions have already be published on bigger animals, but with a lower reproductibility, hard to realize, and time consuming[4,5]. Rat elastase aneurysm model is cheap and feasible with a few practice, with a low mortality[11].

The main limitations of this model is the high frequency of vessel occlusion. The stentgraft, or the aorta easily thrombosed, due to possible intima injuries during stentgraft insertion and clots inside the stentgraft after deployment. Washing carefully the stentgraft is really important before stitching the aorta. Giving aspirin to the rats after the operation could be useful for thrombosis prevention.

Recent studies show a significant percentage of aneurysm growth after endovascular repair in humans[12]. A precise analysis of the different stages of thrombus retraction, hypothesis validations, and lots of graft material tests would be performed on this new murine model.

Disclosures

I have nothing to discole

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