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Induction of autoimmune abdominal aortic aneurysm in pigs - A novel large animal model

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HIGHLIGHTS

- An Experimental study of decellularized aortic xenografts from sheeps implanted into the abdominal aorta in pigs.
- The study shows that it's possible to induce autoimmune AAA with progressive expansion in pigs.
- The induced autoimmune AAAs in pigs where presence already at day 28.
- Intraluminal mural thrombus development also occurred in this study.
- The study also examined the efficiency of SDS with DNase-I as decellularizing detergents on sheep aorta.

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ABSTRACT

Background: Abdominal aortic aneurysm (AAA) is a common disease with a high mortality. Many animal models have been developed to further understand the pathogenesis of the disease, but no large animal model has been developed to investigate the autoimmune aspect of AAA formation. The aim of this study was to develop a large animal model for abdominal aortic aneurysm induction through autoimmunity by performing sheep-to-pig xenotransplantation.

Methods: Six pigs underwent a xenotransplantation procedure where the infrarenal porcine aorta was replaced by a decellularized sheep aorta. In the following 47 days, the AP-diameter of the xenografts was measured using ultrasound once a week. All xenografts were harvested for histological analyses.

Results: All the xenografts formed aneurysms with a mean increase in AP-diameter of 80.98 ± 30.20% ($p < 0.005$). The ultrasound measurements demonstrated a progressive aneurysmal expansion with no sign of halting towards the end of the follow-up period. Histology showed destruction of tunica media and the elastic tissue, neointimal hyperplasia, adventitial thickening with neovascularization, infiltration of lymphocytes and granulocytes, and in some cases intramural haemorrhaging.

Conclusion: We developed a novel large animal AAA model by infrarenal aortic sheep-to-pig xenograph transplantation resulting in autoimmune AAA induction with continuously progressive aneurysmal growth. This model can be used to provide a better understand the autoimmune aspect of AAA formation in large animals.

1. Introduction

Abdominal aortic aneurysm (AAA) is an irreversible dilatation of the abdominal aorta with an anteroposterior diameter of at least 30 mm in adult humans. AAAs are often asymptomatic and discovered as incidental findings by computer tomography imaging for unrelated health problems, or when the AAA ruptures leading to a surgical emergency with a mortality of 50–80% [1,2].

Autoimmunity appears to play a pivotal role in the pathogenesis of AAA as a result of systemic autoimmune responses [1]. Hallmark

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features of autoimmunity in AAAs are the presence of Russell bodies, chronic inflammation with infiltration of oligoclonal T and B cells, elevated cytokines, increased levels of autoantibodies, and an association with HLA molecules [2–4]. Presumed autoantigens have been identified in the aortic wall, such as microfibrillar protein called aortic aneurysm-associated protein-40 (AAAP-40) [2,3]. Several microorganisms that share sequence similarities with AAAP-40 have also been linked to aneurysm development [2–4]. By molecular mimicry, cross-reactivity of epitopes has been suggested to be the mechanism behind the T-cell inflammatory response and the autoimmune induction of AAA. In support, anti-inflammatory and immunosuppressive drugs have already been tested in both animal models and human observational studies, revealing a reduced expansion of AAAs [2,3]. However, the specific targets are still unknown, and further investigation is needed before medical therapy of AAAs can be marketed. Although there are many well established rodent models of AAA [5] the current techniques to induce experimental AAAs in large animals include chemical induction with elastase alone or in combination with collagenase or calcium chloride, mechanical induction with balloon dilatation and surgical induction with stenotic banding or vein patching, or combinations of these [6–8]. These models seem to come to a halt at 75–100% increased dilatations as a sign of healing [7,9]. Based on the hypothesis about the formation of AAA mediated by autoimmunity, the aim of this study was to develop a novel autoimmune AAA model by performing a sheep-to-pig xenotransplantation. Thus, introducing an immune rejection response following implantation of tissue from a foreign animal species [6]; however, the xenograft was decellularized upon implantation to minimize the risk of acute rejection.

2. Materials and methods

The experimental animal study was approved by The Danish Animal Experiments Inspectorate (license no. 2015-15-0201-00523). The experiments were conducted on six female Danish Landrace pigs weighing about 40 kg (range 39.5–41.5 kg).

Infrarenal aortas from female sheep about 180 days old and weighing approximately 40 kg were obtained from a Danish slaughterhouse using semi-sterile conditions, and stored in sterile 0.9% NaCl solution with penicillin-streptomycin (100 U/mL, life technologies) at 4 °C until further processing.

All pigs used were from a specific-pathogen-free herd and evaluated as healthy by a veterinarian prior to inclusion. A special focus on infections diseases were given as this could alter the immunological process after the transplantation, and the donating sheep were similarly inspected and declared healthy by a veterinarian before being euthanized. Moreover, no vascular pathologies were observed during surgery or by histological evaluation of the removed infrarenal aortas".

2.1. Graft preparation

The sheep aortic xenografts were treated in 0.5% SDS with 0.2 mg/mL DNase-I in Tris-EDTA buffer (TE buffer) at room temperature for 24 h with continuous shaking, and then rinsed with sterile 0.9% NaCl. The xenografts were stored in 0.9% NaCl with penicillin-streptomycin at 4 °C until transplantation.

2.2. AAA induction by sheep-to-pig decellularized infrarenal aortic xenotransplantation

The pigs were selected randomly from different litters for surgery by the animal keepers blinded from the kind of study and the selected decellularized sheep graft, which was selected 2–3 days before the transplantation by author MA.

The pigs were anaesthetized as previously described [10]. Preoperatively, a single dose of cefuroxime 1.5 g was administered intravenously.

Through a midline laparotomy the infrarenal aorta was exposed and dissected free from surrounding connective tissue. The lumbar artery closest to the renal arteries was preserved to prevent spinal cord ischaemia. (Fig. 1A). After 5000 IU of unfractionated heparin sodium was administered intravenously, the infrarenal aorta was clamped. An appropriate length of the infrarenal porcine aorta was removed (Fig. 1B) and replaced with the decellularized xenograft by end-to-end anastomosis using 5-0 Prolene sutures (Ethicon LLC, USA) (Fig. 1C and D). Transabdominal ultrasonography (USG) of the xenograft was performed in the longitudinal plane to measure the baseline anteroposterior systolic diameter (AP-0). Finally, the retroperitoneum and laparotomy were closed.

2.3. Postoperative care

Intramuscular injections of Streptocillin (Boehringer Ingelheim, Denmark) 1 mL/10 kg BW, flunixin 100 mg, and buprenorphine 1.8 mg was administered for the first three days as infection prophylaxis and pain relief, respectively.

2.4. Data collection

On postoperative day 8, 13, 22, 28, 35 and 47, the pigs were sedated in order to determine AP-diameter by USG performed by 4 observers. An AAA was defined as an infrarenal aortic AP-diameter of more than 1.5 times larger than AP-0. On postoperative day 47, blood samples were drawn in addition to USG measurements, thereafter the pigs were euthanized with a lethal dose of i.v. pentobarbital 300 mg/mL and the aortic xenografts including some of the porcine native aorta in both ends were harvested for histological and immunohistochemical analyses. Haematological parameters were analysed from the blood samples and compared with 12 healthy age- and sex-matched control pigs as previously described [8].

2.5. Histology

One middle and one distal section from each xenograft were fixed in 10% NBF and embedded in paraffin, along with both proximal and distal sections of the transplant area belonging to the porcine native aorta. The specimens were sliced in 5 μm sections and stained with haematoxylin and eosin (HE), Masson's trichrome (MT, Sigma), Miller's elastic counterstained with Van Gieson (EVG, Atom Scientific), and toluidine blue according to the manufacturer's instructions (Sigma). For immunohistochemistry, the specimens were stained with anti-α smooth muscle actin antibody (α-SMA) (Abcam, UK), and anti-mast cell tryptase antibody (Abcam, UK). All samples were compared to control sections from either the infrarenal sheep aorta prior to decellularization or the infrarenal porcine aorta removed during the transplantation procedure.

2.6. Sample size and statistical analyses

The sample size was based upon a power calculation based using paired *t*-test to be able detect a difference of 50% using the Kloster Study baseline data [8]. The paired two-sample *t*-test was used to compare the mean aortic AP-diameter at AP-0 with postoperative day 8, 13, 22, 28, 35 and 47. The unpaired two-sample *t*-test was used to compare haematology parameters between the experimental animals and controls. We carried out the analyses using Stata 13.0, StataCorp LP, USA. Results are presented as