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Inhibition of early AAA formation by aortic intraluminal pentagalloyl glucose (PGG) infusion in a novel porcine AAA model

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HIGHLIGHTS

- Pentagalloyl glucose impairs the early AAA development in a porcine model.
- Pentagalloyl glucose stabilizes arterial elastic lamellae and preserves their integrity.
- Pentagalloyl glucose can penetrate the arterial wall in large AAA prone arteries from the inside.
- Pentagalloyl glucose, a potential new drug for stabilizing small abdominal aneurysms.

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ABSTRACT

Background: The vast majority of abdominal aortic aneurysms found in screening programs are small, and as no effective treatment exits, many will expand until surgery is indicated. Therefore, it remains intriguing to develop a safe and low cost treatment of these small aneurysms, that is able to prevent or delay their expansion.
In this study, we investigated whether intraluminal delivered pentagalloyl glucose (PGG) can impair the early AAA development in a porcine model.
Methods: The infrarenal aorta was exposed in thirty pigs. Twenty underwent an elastase based AAA inducing procedure and ten of these received an additional intraluminal PGG infusion. The final 10 were sham operated and served as controls.
Results: All pigs who only had an elastase infusion developed macroscopically expanding AAAs. In pigs treated with an additional PGG infusion the growth rate of the AP-diameter rapidly returned to physiological values as seen in the control group. In the elastase group, histology revealed more or less complete resolution of the elastic lamellae in the media while they were more abundant, coherent and structurally organized in the PGG group. The control group displayed normal physiological growth and histology.
Conclusion: In our model, intraluminal delivered PGG is able to penetrate the aortic wall from the inside and impair the early AAA development by stabilizing the elastic lamellae and preserving their integrity. The principle holds a high clinical potential if it can be translated to human conditions, since it, if so, potentially could represent a new drug for stabilizing small abdominal aneurysms.

1. Introduction

It is estimated that the global number of aortic aneurysm related deaths has increased from around a 100.000 in 1990 to around 152.000 in 2013, corresponding to a 52% increase [1].

The risk of developing an abdominal aortic aneurysm (AAA) increases with age, and screening programs have revealed a prevalence of about 3.3–4.9% in European men aged 64–73 (highest in the Caucasian subgroup) [2–5]. The MASS study found that the majority (71%) of these aneurysms were small 3–4.4 cm. A

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definitive surgical treatment (open or endovascular) is in general only recommended for aneurysms with an external diameter ≥ 5.5 cm, aneurysms that expand >1 cm/year or become symptomatic [6,7]. However, since smaller aneurysms (3.5–4 cm) grow on average 2.5–3 mm/year with an exponentially increasing growth rate of about 1.62 mm/year per 1-cm increase in aneurysm diameter, many will eventually reach a size where surgery is indicated. It has been found, that 3.5 cm and 4.5 cm AAAs would take on average 6.2 years and 2.3 years respectively to reach 5.5 cm [7,8].

Despite intensive research into the complex multifactorial etiopathogenesis of this degenerative disease, the exact mechanisms involved are not yet fully understood and no effective medical treatment exits.

Therefore, it remains intriguing to develop a safe and low cost treatment (medically/minimal invasive) of these small aneurysms, that is able to prevent or delay their expansion.

Increased proteolytic elastase and collagenase activity leading to progressive degradation of the aortic walls connective tissue resulting in decreased tensile strength, has been a key early finding [9,10]. In vivo stabilization of infrarenal aortic elastin by means of periadventitially delivered pentagalloyl glucose (PGG) in adult male Sprague-Dawley rats, exposed to $CaCl_2$-mediated aortic elastin injury, has shown to prevent early AAA formation by binding to elastin and thereby preserving the integrity of the elastic lamellae [11]. However, translation of experimental results in rodents to humans has shown difficulties. Consequently, we hypothesized, that intraluminal delivered PGG into the infrarenal aortic segment of our previously described elastase based porcine AAA model in the 30–38 kg weight range [12], also is able to penetrate the arterial wall in large arteries from the inside and stabilize the elastic lamellae, preserving their integrity and hereby impair the early development of AAAs in our model.

2. Materials and methods

Thirty female Danish Landrace pigs in the 30–38 kg weight range were divided into 3 groups of 10. Group A were subjected to balloon dilatation, elastase and a juxtrarenal stenosing cuff (n = 10, mean weight 34 kg (range 31–38 kg)). Group B were subjected to balloon dilatation, elastase, PGG and a juxtrarenal stenosing cuff (n = 10, mean weight 33 kg (range 31–35 Kg)), and control group C underwent a sham procedure (n = 10, mean weight 34 kg (range 30–38 kg)).

2.1. Anesthesia and surgical procedure

Anesthesia was induced by im. injection (mg/kg BW) of 1.25 mg tiletamine, 1.25 mg zolazepam, 0.25 mg butorphanol, 1.25 mg ketamine and 1.25 mg xylazine. After tracheal intubation, the animals were placed in the supine position and ventilated with oxygen 4 L/min and atm. air (1:1, v/v) and anesthesia was extended by continuous intravenous infusion of 10 mg propofol and 25 μg fentanyl per kg BW/h.

With the animals in the supine position a transabdominal ultrasound scan of the infrarenal aorta was performed in both the transverse and longitudinal plane to measure the systolic preoperative external anterior-posterior diameter (AP0).

Through a midline longitudinal laparotomy and a retrocolic prerenal transperitoneal approach the aorta was dissected from the lowest renal artery to the trifurcation, whereafter the lumbars, inferior mesenteric artery and the aorta itself were temporarily clamped. Group C had the clamps removed after 30 min. In group A and B a 2.5 mm arteriotomy in the aorta was made at the level of the inferior mesenteric artery through which a 10 mm × 4 cm high-pressure balloon catheter was placed and inflated for 5 min. Hereafter a curved beaded knop needle was introduced through which 10 ml of porcine pancreatic elastase (Sigma-Aldrich Denmark A/S, E1250-100MG, Type-I ≥4.0 units'/mg protein) was gradually manually infused into the aortic lumen over 30 min. Then a 12 mm × 4 cm high-pressure balloon catheter was introduced and inflated for 5 min. After the second balloon dilatation the first five pigs in group B had 25 MG and the last five pigs in group B had 50 MG of PGG (Penta-O-galloyl-β-D-glucose hydrate, Sigma-Aldrich Denmark A/S, G7548, ≥96% (HPLC)) dissolved in 10 ml of isotonic saline infused into the lumen and flushed for 30 min. Hereafter the arteriotomy was sutured and the aorta declamped. To establish turbulent flow in the infrarenal aortic segment of group A and B, a stenosing nylon cable-tie strap (120 mm × 5 mm) was placed as a cuff around the aorta just below the renal arteries and narrowed until a thrill indicating turbulent flow in the infrarenal aortic segment could be palpated and postoperatively be visualized by Doppler sonography. Finally, the retroperitoneum and laparotomy were closed.

2.2. Postoperative care

Postoperative the animals were monitored the subsequent 28 days. On the 3rd 7th 14th 21st and 28th postoperative days the pigs were anaesthetized and the AP-diameter of the infrarenal aorta was again measured using transabdominal ultrasound. Hereafter the animals were euthanized with a lethal overdose of intravenous phenobarbital and the infrarenal aortic segment was removed and fixed in 10% buffered formalin.

2.3. Histology

The specimens were sliced and stained with routine protocols for human tissue for x100 and x400 microscopy with hematoxylin and eosin stain, Verhoeff's stain for elastin and immunohistochemical staining for human smooth muscle actin (that was found to react satisfactory with tissue from pigs). Actin staining was done in a Ventana autostainer using Actin, Muscle Specific Antibody clone HHF35 from CELL MARQUE (6600 Sierra College Blvd. Rocklin, CA 95677 USA.) that labels smooth and striated muscle cells.

2.4. Statistics

Using mixed models for repeated measures a multivariate analysis of variance (MANOVA) was performed to compare the correlations of the aortic AP-diameters in the three groups over time. In order to determine whether the slopes of the increase in AP-diameter between the groups were comparable Wald chi^2 tests were carried out. One-way ANOVAs were used to compare mean weights between groups. Results were presented as means ± SD and with 95% confidence intervals P-values < 0.05 were considered significant.

3. Results

The three groups (A, B and C) were comparable in terms of mean baseline weight Kg0 ($P = 0.45$) and AP-diameter AP0 ($P = 0.36$). Throughout the experiment the mean weight did not differ significantly between the three groups (Table 1).

The mixed models MANOVA analysis revealed a significant difference in AP-development between the groups ($P < 0.001$) (Table 1).

After 28 days all pigs in group A developed macroscopically AAAs with a mean increase in AP-diameter to 16.26 ± 0.93 mm equivalent to an increase of 57% ± 10.17 SD (Range 31%). In group B the mean increase was lower and equal to 12.17 ± 0.13