Effect of impaired vasa vasorum flow on the structure and mechanics of the thoracic aorta: implications for the pathogenesis of aortic dissection

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Abstract

Objective: To investigate the alterations of structure and mechanical properties of the aortic wall, resulting from impairment of vasa vasorum flow. Methods: Eight healthy Landrace pigs were subjected to interruption of vasa vasorum flow to the upper segment of their descending thoracic aorta. Under sterile conditions, the periaortic tissue was excised and the contiguous intercostal arteries were ligated. Ten sham-operated pigs were used as controls. Fifteen days postoperatively, the animals were sacrificed and their upper descending thoracic aortas were removed. Histology, and collagen and elastin content determination by image analysis technique were performed. Mechanical analysis of aortic strips was carried out with a uniaxial tension device and stress-strain curves were obtained. Results: In contrast to normal aortic walls of the control group, histology of the avascular aortas revealed severe ischemic necrosis of the outer media along with abnormal straightening of the elastin and collagen fibers, without significant collagen and elastin content changes. The borderline between the outer ischemic and inner non-ischemic media was sharp, and an outset of dissection was observed at this point. Mechanical analysis showed that at the same level of strain, the ischemic aorta was significantly stiffer at both low (P < 0.03) and high strains (P < 0.003). Conclusions: Impairment of blood supply to the thoracic aorta leads to abnormal morphology of elastin and collagen fibers of the outer media, resulting in increased aortic stiffness under a wide range of stresses. In the clinical setting, decreased vasa vasorum flow, reportedly occurring in arterial hypertension, may increase the stiffness of the outer media of the thoracic aorta and produce interlaminar shear stresses, contributing to the development of aortic dissection. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Nutrition of the outer layers of the thoracic aorta depends on vasa vasorum (VV) flow, which is reportedly decreased in arterial hypertension and several other conditions [1–8]. It has been long known that impairment of aortic wall blood supply produces medial degeneration and necrosis [3,4], yet its sequelae on aortic elasticity have recently been investigated, only in vivo [1]. The present study aimed to assess the effect of interruption of VV flow on the mechanics of the aortic wall under a wide range of stresses in vitro, and to evaluate the relation between mechanical and morphological alterations.

2. Materials and methods

Eighteen healthy Landrace pigs of either sex, weighing 18.5–21.5 kg, were randomized into two groups. Eight pigs were subjected to surgical interruption of VV flow to their descending thoracic aorta (experimental group), whereas ten pigs had sham-operation (control group). Body weight and gender were similar in both groups.

This investigation was approved by the ethics committee of our institution and conducted in compliance with the European Convention on Animal Care.

2.1. Animal preparation and surgery

Animals were premedicated with intramuscular diazepam (0.25 mg/kg) and atropine sulfate (0.005 mg/kg). General anesthesia was induced with intravenous pentobarbital sodium (30 mg/kg). Supplementary doses were administered throughout surgery to provide steady anesthetic
state. The pigs were intubated, connected to a volume respirator (Harvard Apparatus Inc., South Natick, MA) and ventilated with room air. Continuous pulse oxymetry, electrocardiographic and non-invasive blood pressure monitoring were carried out intraoperatively.

Under sterile conditions, a left thoracotomy was performed through the fourth intercostal space. The lung was retracted downwards, the pleura covering the upper descending thoracic aorta was excised, and the aorta was exposed. In experimental group animals, the periaortic connective tissue, containing the periadventitial VV network, was excised from the origin of the left subclavian artery up to 4 cm distally. Electrocautery was not used and bleeding was controlled with the application of gauze moistened with normal saline at 37°C. The intercostal arteries arising from the stripped portion of the aorta were subsequently ligated at their origin and divided. The completely mobilized part of the aorta was wrapped in a piece of non-porous material (polyvinyl chloride), precluding its contact with the adjacent tissues. In control animals, dressings moistened with normal saline at 37°C were applied on their aortas, but manipulations aiming to impair VV flow were not performed. The chest was closed in layers and the pneumothorax was treated. All animals were normotensive intraoperatively and recovered quickly. Postoperatively, they were actively ambulatory and apparently in good health.

Fifteen days afterwards, all animals were premedicated and anesthetized as described above. Euthanasia was induced with large doses of pentobarbital sodium, and the chest was opened through a median sternotomy. The left pleural cavity was entered and the upper descending thoracic aorta was excised with extreme care to avoid damage of the aortic wall.

2.2. Histological studies

A 1-cm wide longitudinal segment of the excised aorta was cut and promptly fixed in 10% buffered formalin. All specimens were embedded in paraffin using standard techniques. Tissue blocks were sliced into 5-μm sections and stained with hematoxylin-eosin, Verhoeff’s elastica for elastin and Masson’s trichrome for collagen. Glass slides were coded and assessed blindly.

Elastin and collagen percentage content of the aortic wall was measured by computer-assisted image analysis in appropriately stained cross sections, as described elsewhere [1,9]. In the control group, image analysis was performed in 32 randomly selected images of the entire media on each glass slide. In the experimental group, however, because of marked histological differences between the outer and inner media, 16 randomly selected images from each region were analyzed, and the total elastin or collagen content of the media $C$ was calculated as:

$$C = \frac{C_{\text{outer}}l_{\text{outer}} + C_{\text{inner}}l_{\text{inner}}}{l_{\text{outer}} + l_{\text{inner}}}$$

where $C_{\text{outer}}, C_{\text{inner}}$ were the contents of the respective medial regions, and $l_{\text{outer}}, l_{\text{inner}}$ their thickness. The borderline between these regions was quite sharp and their thickness was readily measured by means of the image analysis software (SigmaScan, SPSS Inc., Chicago, IL).

2.3. Mechanical analysis

From the remaining aortic specimen, strips of fixed dimensions were obtained, and immediately subjected to stress–strain analysis with an automatic uniaxial tension device (Vitrodyne V1000, Liveco Inc., Burlington, VT). During the test, all specimens were submerged into a saline bath at a constant temperature of 37°C.

Prior to measurements, each strip was subjected to ten successive tensile cycles, so as to minimize the effect of viscoelastic phenomena, and, hence, obtain constant and reproducible stress–strain curves [10]. Measurements were stored in a computer. Considering the aortic wall incompressible, stress $\sigma$ and strain $\gamma$ were automatically calculated as:

$$\sigma = \frac{F l}{w_0 h_0 l_0}$$

and

$$\gamma = \frac{1}{2} \left( \frac{1}{l_0} - 1 \right)$$

where $l_0, w_0$ and $h_0$ were the initial length, width and thickness of the strip, $l$ the deformed length, and $F$ the longitudinal force applied to the strip. Thickness $h_0$ was measured via a laser beam micrometer, with a nominal error of ±1 μm (LS-3100, Keyence Corp., Osaka, Japan). Parameters $l$ and $F$ were measured by the tension device with a frequency of 50 Hz.

Since the aortic wall elasticity is highly nonlinear [10], curve fitting was employed for the evaluation of the stress–strain curves. The curves were plotted as the slope of the curve versus stress, resulting in bilinear graphs. The two distinct linear parts of these graphs corresponded to well-defined regions of the stress–strain curves, referred to as the low-strain and high-strain region. Curve fitting was performed for these regions in terms of functions $\sigma = A e^{B \gamma}$ and $\sigma = C e^{D \gamma}$, respectively (A, B, C, and D are curve-fitting parameters). It was proven that, at the same level of strain, and for $B = D$, the ratio of elastic moduli of two different aortic specimens equals the ratio of parameters A and C at the low-strain and high-strain regions, respectively, whereas at the same level of stress, the ratio of elastic moduli equals the ratio of $B$ and $D$ at the low-strain and high-strain regions, respectively. Parameters A, B, C, and D were calculated and, hence, aortic elasticity alterations were quantified.
2.4. Statistical analysis

Results are expressed as mean ± standard error of the mean. The unpaired Student’s t-test was used to estimate differences between groups regarding the curve-fitting parameters, and the elastin and collagen contents of the specimens. A P-value less than 0.05 was considered statistically significant.

3. Results

3.1. Morphological outcomes

On postmortem, in contrast with the apparently normal aortas of the control animals, the avascular aortas were enclosed in a dense fibrous sheath. However, polyvinyl chloride had effectively prevented involvement of the aortic wall in postoperative fibrosis, and specimens were removed easily. On gross examination, the wall of avascular aortas was not solid but evidently presented a fissure dividing the media into two layers. A mild traction was enough to separate these layers, resulting in typical aortic dissection (Fig. 1).

The microscopical structure of aortas of sham-operated pigs was normal. In experimental group aortas, the VV network was entirely removed with no evidence of neovascularization. Interrupted blood supply resulted in severe ischemic necrosis of the outer media, microscopically manifested as complete loss of smooth muscle cells. Major alterations of the elastin and collagen fibers architecture were also evident (Fig. 2a). The fibers had lost their normal sinuous pattern and appeared somewhat thinned and focally fragmented (Fig 2b). Thin interlaminar elastic fibers were sparse and adjacent elastic lamellae, although closely positioned to each other, presented a rather loose interconnection. Besides, absence of leukocytic infiltration was observed, substantiating the adequacy of surgically induced ischemia. Despite these dramatic alterations of the outer media, the inner media appeared normal. The borderline between these two regions was sharp, and in several sections a microscopical outset of dissection was observed at this point (Fig. 2a).

Image analysis revealed no significant difference in the elastin and collagen content of the outer and inner media of the avascular aortas. Likewise, the total elastin and collagen content was similar in the aortas of both groups (Table 1).

3.2. Mechanical analysis

The cumulative stress–strain diagrams indicated that the ischemic aortic wall was significantly stiffer (Fig. 3). Calculation of curve fitting parameters showed that parameter $A$ increased from $(0.117 \pm 0.012) \times 10^{6}$ g/m$^2$ in normal to $(0.205 \pm 0.025) \times 10^{6}$ g/m$^2$ in ischemic aortas ($P = 0.03$), whereas the value of $B$ did not change significantly ($(2.668 \pm 0.110) \times 10^{6}$ g/m$^2$ and $(2.895 \pm 0.086) \times 10^{6}$ g/m$^2$, respectively). Similarly, parameter $C$ increased from $(0.027 \pm 0.005) \times 10^{6}$ g/m$^2$ in normal to $(0.112 \pm 0.016) \times 10^{6}$ g/m$^2$ in ischemic aortas ($P = 0.003$), whereas parameter $D$ ($(3.669 \pm 0.102) \times 10^{6}$ g/m$^2$ and $(3.269 \pm 0.192) \times 10^{6}$ g/m$^2$, respectively) exhibited no statistical difference between the two groups.

These findings suggested that at the same level of stress, normal and ischemic aortas were equally stiff in both
regions of the stress–strain curve, since the values of parameters $B$ and $D$ were not significantly different in the two groups. On the contrary, at the same level of strain, ischemic aortas were significantly stiffer than normal, their elastic modulus approximating two and four times that of the control group at the low-strain and high-strain region of the stress–strain curves, respectively ($A_{\text{ischemic}}/A_{\text{normal}} = 1.752$ and $C_{\text{ischemic}}/C_{\text{normal}} = 4.148$).

4. Discussion

In this study, the effect of impaired VV flow on the elastic properties of the thoracic aorta was investigated in vitro. Stefanadis et al. have shown an increased stiffness of the ascending thoracic aorta of mongrel dogs in vivo 15 days after removal of periaortic VV [1]. Our results are concordant with this finding. Moreover, the current analysis
demonstrated that increased stiffness occurs not only within the range of pressures encountered in vivo but throughout a wide range of stresses from zero, up to nearly the level of material failure. These mechanical alterations were associated with dramatic structural changes of the media.

4.1. Morphological changes and their relation to aortic stiffening

It is known that surgically induced impairment of VV circulation results in degenerative alterations of the aortic media [3,4]. In this study, however, there were two important technical innovations: (i) combination of intercostal arteries ligation with stripping of periaortic VV network, both producing aortic wall ischemia, and (ii) wrapping of the avascular aorta with a nonporous material. The former aimed at extensive interruption of VV flow, including collateral circulation from the inferior thyroid arteries and periadventitial vessels of the aortic arch [2]. The latter aimed at prevention of periaortic fibrosis and aortic wall neovascularization, which reportedly restores blood flow up to normal levels within 2–4 weeks [11]. The attempt for such prevention has been successful, since active proliferation of fibroblasts and capillaries, and newly formed collagen fibers in the adventitia [3] were not observed in this study. The above-mentioned innovations eventually resulted in severe ischemia, not limited to the mid portion of the media but rather involving the outer two thirds of it, without regenerative changes in the necrotic regions [3,4]. As the utmost manifestation of aortic necrosis, gross and microscopic evidence of aortic dissection were described herein.

The mechanical analysis demonstrated a bilinear response of the aorta, which is a typical feature of passive arterial mechanics, due to the specific mechanical characteristics of aortic constituents, namely elastin and collagen [12–14]. The elastic behavior of the aorta depends upon the amounts of elastin and collagen, yet the present histological analysis demonstrated no significant change in collagen and elastin content resulting from ischemia. However, fibers were uncrumpled and straightened in the necrotic region. In fact, this distorted morphology was responsible for the increased stiffness of the ischemic aorta. Straightening raised the fraction of fibers supporting the wall stress at different levels of strain and, consequently, increased the elastic modulus at the same level of stress, since the degree of straightening of the fibers was evidently identical for the same value of stress.

4.2. Aortic dissection: a new perspective of the role of VV in its pathogenesis

VV are most abundant in the ascending aorta and arch, precisely the segments most susceptible to the development of aortic dissection. However, their role in the pathogenesis of dissection remains obscure. In view of the findings of this study, it was speculated that a mechanical aortic defect might underlie the pathogenesis of dissection.

| Elastin and collagen percentage content of the aortas, as resulted from the image analysis |
|---------------------------------|-------------------|-------------------|-------------------|-------------------|
|                                | **Elastin**       | **Collagen**      | **Elastin**       | **Collagen**      |
| **Experimental group**         | **Outer necrotic media** | **Inner normal media** | **Total content** | **Experimental group** | **Control group** |
| Outer necrotic media           | 39.9 ± 6.3%       | 45.5 ± 3.5%       | Not significant   | 41.6 ± 2.5%       | 45.2 ± 3.2%       | Not significant   |
| Inner normal media             | 29.7 ± 5.4%       | 31.8 ± 2.6%       | Not significant   | 32.0 ± 2.8%       | 32.4 ± 4.0%       | Not significant   |

Table 1

Fig. 3. Cumulative stress–strain diagrams of the experimental and control groups. The curve corresponding to the ischemic aorta is shifted to the left and its slope at all levels of strain is higher, indicating that the aortic wall became stiffer after the interruption of blood supply. The solid and dashed vertical lines divide the ischemic and normal stress–strain curves, respectively, into their low-strain (related to parameters A and B) and high-strain region (related to parameters C and D).
Arterial hypertension, indisputably the chief predisposing factor of dissection [15,16], is accompanied by hypertrophy and hyperplasia of aortic smooth muscle cells [17], and increase of oxygen consumption [18]. On the other hand, chronic hypertension limits the vasodilator capacity of aortic VV [7]. Under these circumstances, VV flow most probably fails to meet with the increased metabolic requirements of the aortic media, resulting in some degree of ischemia. In hypertensive crises, ischemia is likely to be aggravated because VV constrict, despite the further increased metabolic needs [5,8]. As shown in this study and others [3–5], aortic ischemia is probably limited to the outer medial layers, the inner media being adequately nourished by diffusion from the lumen [4,5]. Based on the current results, it is reasonable to presume that ischemia of the outer media is accompanied by mechanical alterations, namely stiffening. If this is the case, the thoracic aortic media of hypertensive patients can be considered as a composite material consisting of a sufficiently nourished inner region with normal elasticity and an outer ischemic region with increased stiffness. Owing to the different elastic moduli of the two regions, interlaminar shear stresses are likely to develop at their borderline [19]. Histology showed this borderline to be quite sharp. Interlaminar stresses may eventually lead to detachment of the layers and aortic dissection. This pathogenetic model supports the concept of the aorta as a functional organ and emphasizes the role of mechanics in the comprehension of aortic pathophysiology.

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References


Appendix A. Conference discussion

Dr M. Turina (Zurich, Switzerland): You selected 15 days as the time of your study. I have two questions. What led you to the choice of 15 days? Dissection is a continuous process; there is a change in the proportion of the collagen and elastin in the wall, and there is a repair process. Do you have any observations earlier and later than 15 days?

Dr Angouras: A few years ago, in our laboratory, Stefanadis and coworkers investigated in vivo the mechanical alterations of the thoracic aorta 30 min and 15 days after vasa vasorum removal [1]. Based on their histological findings, we felt that 15 days are sufficient for structural alterations to fully develop in the avascular aortic wall. Moreover, we wanted the results of our in vitro mechanical analysis to be comparable to the in vivo data of this previous study and, therefore, we sacrificed the animals 15 days postoperatively. Both studies showed a significant increase of aortic stiffness after impairment of vasa vasorum flow. The advantage of our in vitro analysis was that the elasticity of the aorta was examined under stresses not limited within the physiological values (i.e. between diastolic and systolic arterial pressure) but ranging from zero up to a level approaching material failure. Throughout this wide range, the aortic wall was proved to be significantly stiffer as a result of ischemia.

Regarding your second question, I am afraid we have no observations earlier or later on. Under the specific experimental conditions of this study, however, a repair process of the necrotic aortic wall was not expected, since such a repair presupposes restoration of blood supply, and migration of inflammatory cells and fibroblasts to the necrotic area. As pointed out in the article, wrapping of the avascular aortas of our animals with a non-porous material effectively prevented the development of neovascularization. Active proliferation of capillaries and fibroblasts, and newly formed collagen fibers in the adventitia were not observed. Thus, a change of the elastin and collagen percentage content of the media as a result of repair of the ‘injured’ tissue, does not seem very likely in this particular setting.