Does focal destruction of the thoracic aorta wall by *Staphylococcus aureus* lead to the development of infected aneurysms? An experimental study

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Received 3 May 1999; received in revised form 26 July 1999; accepted 25 October 1999

Abstract

Objective: The infrequency of infected aneurysms suggests that either infection of segments of the aortic wall is uncommon, or that infections do not always lead to infected aneurysm formation. The purpose of the study was to determine whether focal *Staphylococcus aureus* infection of aortic wall segments leads consistently to the development of infected aneurysms and to evaluate the segments in which infection did not lead to the infected aneurysm formation.

Methods: Twenty pigs were inoculated with 0.1 ml of a *Staphylococcus aureus* inoculum in three segments of the thoracic aorta wall (study group). In another 10 pigs, 0.1 ml of saline solution was injected in three segments of the thoracic aorta wall (control group). Study group: histological abnormalities and bacterial culture of the inoculation sites were evaluated at 10 days (*n* = 5 pigs), 30 days (*n* = 5 pigs), and 90 days (*n* = 10 pigs). Control group: histological abnormalities were evaluated at 10 days (*n* = 5 pigs) and 90 days (*n* = 5 pigs).

Results: Study group: infected aneurysms developed in only two animals killed at 30 days. At 90 days, destruction of the elastic tissue, scar tissue and neointima formation were found in all the aortic segments studied. Control group: no significant changes were found in any of the segments evaluated.

Conclusion: In our experimental model, acute local infection by *S. aureus* caused the development of infected aortic aneurysm in only 10% of the animals. In the remaining 90%, healing of the site of infection followed resolution of the infection. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: *Staphylococcus aureus*; Infected aneurysms; Infection; Healing

1. Introduction

Infectious endarteritis generally is the consequence of direct action by infectious agents on the vessel wall which produces a necrotizing inflammation of the segment involved. Most of the necrosis is the consequence of protease release from neutrophils. Histological findings in the wall of the affected segments include medial necrosis, abscess formation, elastic-tissue destruction, abundant polymorphonuclear infiltrate, and presence of the causal microbial agent [1–4]. These lesions may lead directly to vascular erosion and rupture, or to the formation of infected aneurysms (IA) also known as mycotic aneurysms [1–4]. In the post-antibiotic era, one of the dominant infecting organisms responsible for the development of IA is *Staphylococcus aureus* [1–4]. *Staphylococcus aureus* (*S. aureus*) can enter the vessel wall via the lymphatic vessels or vasa vasorum from an adjacent infectious process [5], and appears to have a unique tropism for the vascular endothelium [6]. *Staphylococcal* pathogenesis involves the production and secretion of toxins that damage host cells and enzymes that degrade tissue components [7]. In monocytes, *S. aureus* triggers dose-dependent cytokine production [8] and protease production, which may be responsible for elastic tissue destruction [9,10]. *S. aureus* infections often occur in human beings, all healthy adults have *S. aureus* alpha-toxin antibodies [11], and low-grade *S. aureus* bacteremia occurs even with subcutaneous infections [12].

Clinical studies suggest that once the infection of the aortic wall is established, it continues a destructive course leading to IA formation and/or rupture of the wall of the segment involved, which cannot be eradicated without surgery [2–5]. Nevertheless, clinical studies show that all the IA and wall ruptures resulting from infectious aortitis are caused by an infectious agent, but they shed no light on whether focal destruction of the wall of a vessel by an acute
infection invariably leads to wall rupture or aneurysm formation. Although it seems plausible that infection would lead always to IA formation and/or wall rupture, the infrequency of IA suggests that either focal infection of segments of the aortic wall is uncommon, or that such infections do not always lead to IA formation. The purpose of our study was to evaluate the frequency of IA development after direct infection by \textit{S. aureus} of segments of the thoracic aortic wall, and to evaluate the intermediate-term evolution of the segments that did not develop IA after infection. We directly inoculated three sites of the wall of the thoracic aorta of normolipemic domestic pigs with a 0.1-ml inoculum of \textit{S. aureus} at a concentration of 0.5 on the McFarland scale. Histological changes in the aortic wall and bacterial culture of the inoculated segments were evaluated 10, 30, and 90 days after inoculating \textit{S. aureus}.

2. Materials and methods

Thirty normolipemic domestic pigs weighting 20–25 kg were used in the present study. All animals received humane care in compliance with the ‘European Convention on Animal Care’. The study was approved by the Hospital ethics committee. An inoculum of \textit{S. aureus} was prepared at a concentration of 0.5 on the McFarland scale, which corresponds to $1.5 \times 10^8$ colony-forming units (CFU)/ml. The strain inoculated was \textit{S. aureus} group II 3A phage 55.

2.1. Study protocol

2.1.1. Study group \((n = 20)\)

Twenty pigs were injected with 0.1 ml of the inoculum of \textit{S. aureus} at a concentration of 0.5 on the MacFarland scale in three different sites of the thoracic aorta. Five of these animals were killed at 10 days, five were killed at 30 days, and another 10 pigs were killed at 90 days.

2.1.2. Control group \((n = 10)\)

This group differed from the study group in that 0.1 ml of saline solution was injected into the thoracic aortic wall segments. Five of these animals were killed at 10 days and another five at 90 days.

2.1.3. Experimental model

The animals were sedated with ketamine (12 mg/kg weight), then intubated and ventilated with nitrous oxide, oxygen and isoflurane to maintain anesthesia, as confirmed by the absence of the limb withdrawal reflex. During anesthesia, arterial gasometric measurements were made and systemic blood pressure was monitored continuously. A left lateral thoracotomy was made in the third or fourth intercostal space and the parietal pleura was opened. The thoracic aorta was clamped laterally and an incision was made in the clamped aorta. The edges of the incision were separated in order to provide a direct view of a sterile needle (25G 5/8") as it was introduced into the vessel wall without injuring the endothelium from one of the edges of the incision. Then, 0.1 ml of bacterial inoculum or saline solution was injected with a 1-ml syringe. The needle was withdrawn and the edges of the incision were closed by continuous suture (non-absorbable monofilament suture). The second and third injections were made in the middle third and distal third of the thoracic aorta using the same procedure. The thoracotomy was closed and negative pressure was applied to the chest cavity. None of the animals received antibiotics before or after the surgical intervention. All animals survived until the day scheduled for death. None showed deterioration or any sign of poor general condition, and all fed normally. For 3–4 days after surgery, six pigs had mild fever (maximum rectal temperature 38.5°C), which remitted without treatment.

On the scheduled day, pigs were anesthetized and a left lateral thoracotomy was made. After careful dissection of the descending aorta, the animals were killed by injecting 40 mEq of potassium chloride in the left atrium to induce cardiac arrest. The respirator was disconnected and the descending aorta was sectioned between the origin of the left subclavian artery and the diaphragm. The sectioned aorta was washed with sterile saline solution. The sites of inoculation or saline solution injection were located by the non-absorbable suture. One of the three sites of \textit{S. aureus} inoculation was sectioned and sent in sterile tubes for culture of the inoculation zone. The rest of the thoracic aorta was fixed in 10% formalin for at least 24 h. The two sites of \textit{S. aureus} inoculation and part of the healthy aorta were sectioned into 3-mm rings for embedding in paraffin and histological examination. In the control group, the entire thoracic aorta was fixed in formalin and then sectioned into 3-mm rings and embedded in paraffin. The pathologists were blinded. In every aortic ring, 5-micra cross-sections were stained with hematoxylin and eosin; Masson trichrome for collagen fibers, fibrin and nuclei; and Movat pentachromic II. The total circumference of the vessel, length of elastic destruction, total wall thickness, diameter of the normal and infected segments and medial and neointimal thickness were measured on each section by morphometric method. The thickness of the vascular wall was the distance between the external elastic layer and the endothelium, the thickness of the media was the distance between the internal and external elastic layers, and the thickness of the neointima was the distance between the internal elastic layer and the endothelium. The length of elastic destruction was expressed as a percentage of the total vessel circumference. The thickness of the media and neointima was expressed as a percentage of total vessel thickness. Aneurysms was defined as a focal dilatation of the aorta involving an increase in diameter of at least 50% as compared with the normal artery above it [13]. Significant circumferential elastic tissue destruction was considered the destruction of the elastic fibers for more than 1500 µm [10]. The variables (percent of circumferential extension of the lesion and intimal/medial thickness ratio) for the control
group and the study group were compared using the Student’s t-test. A value of $P < 0.05$ was considered statistically significant.

3. Results

Table 1 shows the mean values and standard deviation of the percent circumferential extension of the lesion and the intimal thickness/medial thickness ratio.

3.1. Histological study

3.1.1. Study group

At 10 days ($n = 10$ segments), the necrosis caused by 

S. aureus

produced destruction of the media and its elastic tissue. An inflammatory reaction consisting mainly of polymorphonuclear giant cells and groups of polymorphonuclear cells was visible around the necrosis zones (Fig. 1). The inflammation extended to the adventitia. The intima showed diffuse thickening, inflammatory infiltrates, and edema. All bacterial cultures were positive and 

S. aureus

phae group II 3A 55, was identified. The histological findings at 30 days ($n = 10$ segments) were similar to those seen at 10 days, except that the media showed a more chronic inflammatory infiltrate and the onset of fibrosis replacing the zone of necrosis. Aneurysmal dilatation was found in the inoculated aortic segments of two of the five pigs killed at 30 days. The inner diameter of the aneurysmal dilatation was almost 50% of the diameter of the vascular lumen ($5 \pm 1$ vs. $11 \pm 1$ mm). The bacterial culture was positive for one pig with aneurysmal dilatation and negative for the others four.

At 90 days ($n = 20$ segments) the media showed scar tissue formed by poorly structured collagen (Fig. 2). The adventitia and intima presented fibrosis and thickening (Fig. 2). Significant circumferential elastic tissue destruction was found in all the aortic segments studied, but no aneurysmal dilatation was found in any case. All bacterial cultures were negative.

The circumferential extension of vascular injury, which involved almost one fourth of the vessel circumference (Fig. 3), was similar in all 20 pigs (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Evolution (days)</th>
<th>CEN (%)</th>
<th>IT/MT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>$5.9 \pm 1.7$</td>
<td>$3.1 \pm 1.3$</td>
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<tr>
<td>Study</td>
<td>10–30</td>
<td>$23.7 \pm 3.2^*$</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>90</td>
<td>$5.1 \pm 1.2$</td>
<td>$2.8 \pm 1.6$</td>
</tr>
<tr>
<td>Study</td>
<td>90</td>
<td>$22.4 \pm 2.4^*$</td>
<td>$41.3 \pm 23.8^*$</td>
</tr>
</tbody>
</table>

*CEN, circumferential extension of necrosis; IT/MT, the intimal thickness/medial thickness ratio. *Significant differences versus controls. *There was no intimal thickness but only extensive intimal necrosis. For this reason measurements are not reported.

3.1.2. Control group

At day 10, a small zone of medial injury (Table 1) was observed accompanied by an exiguous inflammatory reaction and minimal neointimal formation. No significant adventitial changes were visible. At day 90, the small zone of medial injury was healed and the intima did not show significant changes. No significant circumferential elastic tissue destruction developed (Table 1) in any of the segments evaluated ($n = 15$ segments at day 10 and $n = 15$ segments at day 90).

4. Discussion

4.1. Experimental model

We used 

S. aureus

as the infecting organism because one of the dominant infecting organisms responsible for IA formation is 

S. aureus

[2–5]. The focal aortic infection was produced with an inoculum at a concentration of 0.5 on the MacFarland scale because this concentration ensures
that the bacteria remain alive and viable and that the tissue at the site of inoculation will not be massively destroyed by bacterial growth and multiplication. A limitation of our experimental model may be that it does not reproduce the

Fig. 2. At 90 days, scar tissue formation in the media by poorly structured collagen. Internal elastic lamina (arrow) disruption. Fibrosis and thickening of the intima. Movat’s pentachrome stain. E, endothelium.

Fig. 3. Light microscopy photograph of Masson’s trichrome (A) and hematoxylin and eosin stain (B): circumferential extension of necrosis at 30 days after infection. (A) Infected aortic aneurysm. (B) Without aneurysmal dilatation.
causes of IA formation described in humans beings [3]. Because focal infection of the vessel wall is the final step of the three different causes – septic emboli, extension via vasa vasorum or lymphatic vessels or hematogenous seeding of the aortic wall during bacteriemia, of IA formation [3], and previous studies shed no light on whether focal infection invariably leads to IA formation, we directly inoculated segments of the aortic wall with S. aureus. The evolution of these infected segments has been evaluated. In the study group the histological findings at 30 days were similar to those seen at 10 days, for this reason in the control group histological evaluation was made at 10 and 90 days.

4.2. Short-term evaluation: IA formation

The differentiation of IA was made on the basis of histological findings [1–4] and a culture in which the causal germ was identified. Saline solution per se did not induce destruction of the elastic tissue [10], therefore, the small medial injury in the control group seemed to be the result of the trauma of saline solution injection. As the small lesion has been caused by injecting sterile saline solution, and particularly even frank IA can yield negative cultures and a positive culture alone does not justify the diagnosis of mycotic aneurysm [1,5], we did not make cultures of the control group. The inoculum of S. aureus at a concentration of 0.5 on the MacFarland scale produced a lesion with a circumferential extension almost three times greater than that produced in the control group (Table 1), which suggests that the production and secretion of toxins by S. aureus and the production of cytokines and proteinases by activated monocytes and lymphocytes [8,10,14] could be responsible for the major necrosis of the media and elastic tissue destruction. The histological findings in the animals killed at 10 and 30 days (n = 10 pigs), were similar to those reported in infected aortic aneurysms in humans [1–4]. For the above reasons, it could have been expected that IA would form in all the infected segments in the acute phase of infection. Nevertheless, wall rupture did not occur in any case and infected aortic aneurysms were found only in the infected segments of two of the pigs killed at 30 days. Although culture was negative in one of the pigs, we consider that the aneurysms seen in this pig are infected because the histological findings in the wall of the affected segments include medial necrosis, abscess formation, and abundant polymorphonuclear infiltrate. It is known that in some cases cultures of IA may be negative.

Why the infection did not lead to IA formation in the rest of the animals could not be determined from this study. One explanation could be differences in blood pressure. In our study blood pressure was recorded only twice, when the animals were anesthetized. Therefore, we do not know if the formation of an infected aortic aneurysm in these two pigs could have been triggered by episodes of high blood pressure or if they occurred under stable blood pressure conditions. It is likely that the proportion of IA formation would have been different if the mild focal infection had been produced in the wall of an artery with fewer medial lamellar units than the thoracic aorta. Our results suggest that extensive focal destruction of the vessel wall does not always lead to IA formation.

4.3. Intermediate-term evaluation

At 90 days, the infection and removal of necrotic tissue from the injured zone had concluded and the inoculated segment was healed. As a consequence of the incapacity of the adult aorta to synthesize elastin [15], the healed zone had no elastic tissue. Elastin is the primary aortic wall component for the elastic recoil of the artery. If elastin is destroyed, collagen is continuously exposed to expansive force of intraluminal blood pressure, which can cause collagen failure [16] and significant arterial dilation [17]. Likewise, neointimal formation was apparent in these segments. It is well known that neointimal formation is the most important repair mechanism in the vessel wall. In view of earlier findings [18,19], it can be postulated that smooth muscle-like cells stimulated by growth factors released by damaged smooth muscle cells regained their proliferative phenotypic characteristics and produced the fibrous tissue constituents of the intima [18,19].

It is well known that bacterial infection can occur in pre-existing degenerative aneurysms. Likewise, aneurysm formation depend on collagen loss, the presence of exacerbating factors and the time over which these factors operate [4,9,10,13,17,20]. In our study we did not find aneurysm formation at 90 days, but, in view of previous findings [4,9,10,13,17,20], it could be suggested that at least in some of the segments of the aorta in which infection is followed by zone of fibrosis, the possibility of large aneurysms formation cannot be excluded.

5. Conclusion

Although focal infection by S. aureus of segments of the wall of the thoracic aorta caused the destruction of almost 25% of the vascular wall circumference, IA formed in hardly 10% of the segments. In the remaining 90%, segment healing followed cure of the infection.

Acknowledgements

We thank Pilar Denavas Sanchez and Ana Gonzalo Lazaro for their technical assistance.

References