Staphylococcus aureus mediastinitis and sternal osteomyelitis following median sternotomy in a rat model

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Objectives: Median sternotomy (MS) wound infections are severe complications causing high morbidity and mortality after cardiac surgery. We aimed to develop a new Staphylococcus aureus mediastinitis and sternal osteomyelitis model in rats that can be used to evaluate the efficacy of new antimicrobial treatments.

Methods and results: A complete MS wound was induced in anaesthetized rats. S. aureus was injected into the sternum. Kinetics of bacterial growth in the sternum (10^7 cfu/sternum) was assessed for histopathology and bacterial counts. A non-infected MS group served as a control. To evaluate antibiotic efficacy, 5 days of intraperitoneal vancomycin therapy (50 mg/kg, twice a day) was initiated 24 h following bacterial challenge. Macroscopic and histological examination confirmed that infection resulted in sternitis and mediastinitis. S. aureus bacterial counts in the sternum were inoculum-dependent, and it was proven that infecting rats with an inoculum of 10^7 cfu/sternum induced mediastinitis and sternal osteomyelitis. At this inoculum, bacterial counts in the infected sternum increased with time, reaching a maximum level of 2^+++++1310^7 cfu/g of sternum 8–12 days post-infection and then decreased with time to 2×10^4 cfu/g of sternum 20 days after infection. Histological changes paralleled bacterial counts. Vancomycin administration showed a protective effect against induction of sternal osteomyelitis; sternums from vancomycin-treated rats showed a significant decrease in S. aureus counts by 0.72±0.35 log cfu/g compared with untreated controls (P = 0.0162).

Conclusions: This new rat model of S. aureus sternal osteomyelitis and mediastinitis allows quantitative measurement of bacterial counts in the sternum. This model is reproducible and simple and thus suitable for the evaluation of new antimicrobials and new treatment modalities in MS infections.

Keywords: experimental model, infection, vancomycin, S. aureus, antibiotic treatment

Introduction

Deep sternal wound infections pose a serious problem in cardiac surgery despite continuing efforts to improve perioperative conditions. Mediastinitis and sternal osteomyelitis remain the most dreaded complications after median sternotomy (MS) incisions and were reported to occur in 0.5% to 8% of patients undergoing cardiac surgery.1–6 These complications are often associated with significant morbidity and mortality rates of up to 45%,1–6 with prolonged hospitalization3,4 and additional surgical procedures, as well as prolonged antibiotic therapy and its inherent high costs.5,6 Most MS wound infections are related to the host or to various technical factors and less to infectious outbreaks, with both endogenous and exogenous pathways having been found to play a role in their occurrence.7 Staphylococcus aureus is the most common pathogen isolated from MS wound infections after cardiac surgery as well as from bacteremia blood cultures.8–11 Epidemiological studies point to an increasing trend in antibiotic resistance, with the appearance of progressively more cases of methicillin-resistant S. aureus (MRSA) strain infections, causing an increase in mortality, duration of hospitalization and hospital charges.7,10–13 Moreover, an ageing and more morbid cardiac surgery patient population further increases the incidence and severity of MS wound infections.10–13 Thus, new antimicrobials or treatment modalities with increased efficacy are being sought. There is a concomitant need for an animal model that

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Materials and methods

Animals and experimental conditions

Male rats, 8 weeks of age, weighing 250–270 g (Harlan Laboratories, Rehovot, Israel) were housed one rat per cage in a central animal research facility. The facility maintains an environment of controlled temperature and relative humidity, with a 12 h light/dark cycle. Rats were supplied with sterile bedding, standard food and water, and were acclimatized for 10 days before each experiment. Acetaminophen (0.25 mg/mL) in the drinking water was used as a post-operative analgesic. The animals were individually labelled and weighed prior to each experiment, and were sacrificed by carbon dioxide at its termination. All procedures, care and handling of the animals were reviewed and approved by the Institutional Animal Care and Use Committee at the Tel-Aviv Sourasky Medical Center.

MS model

Prior to surgery, the rats were anaesthetized by an intraperitoneal injection (0.5 mL) of a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). The hair on their chest was thoroughly removed by a razor. Each animal was secured in a supine position and the chest incision was opened. The experiments were typically terminated 30 days after inoculation of bacteria. The animals’ survival was assessed daily, and the chest wounds were examined daily for induration, oedema, purulence or dehiscence. The appropriate S. aureus infecting dose for inducing osteomyelitis was determined by performing MS incisions on rats (in groups of 5–8) followed by injection of 1 × 10^3, 1 × 10^4, 1 × 10^5 or 1 × 10^6 cfu/sternum. Ten days after MS and infection, the animals were sacrificed and their sternal bone was removed aseptically, weighed and homogenized. Quantitative bacterial counts of homogenates and histopathology examination for osteomyelitis were determined/ performed for each specimen and compared with the uninfected group of animals that had undergone an MS incision.

Sternal S. aureus growth kinetics

Once the optimal infecting inoculum of S. aureus had been chosen, the kinetics of S. aureus bacterial growth in the sternum was studied by determining S. aureus bacterial counts in the sternum at different times after MS infection. Twenty rats with MS incisions were injected with 1 × 10^7 cfu/sternum. Four rats were sacrificed on post-operative days 2, 5, 8, 12 and 19 after MS wound infection. The chest incision was opened and the sternal area exposed. The sternum was photographed and documented for abscess formation and necrosis, and quantitative bacterial cultures of the sternum and histopathology were performed.

Antibiotic treatment

The infected MS wound was challenged with antibiotic treatment. The 20 rats with MS incision were divided into three groups. The first group (n = 8) included S. aureus-infected (1 × 10^5 cfu/sternum) MS incisions and treatment with systemic vancomycin, which was given intraperitoneally 50 mg/kg every 12 h for 5 days starting at 24 h after MS incision and infection. The second group (n = 8) included controls with infected MS incision that received placebo injections of phosphate-buffered saline (PBS) according to the same regimen as the vancomycin-treated group. The third group (n = 4) had undergone MS incision with no infection and no treatment. The experiment was performed twice and 12 animals were included in the analysis. Quantitative bacterial cultures of the sternum and histopathology were performed.

Histopathology assessment

Histopathology examination was performed on all sacrificed rats to assess the infection process of the bone and the surrounding soft tissue. The sternum of each animal was removed in a sterile manner and fixed in 10% formaldehyde solution. Cross-sections were taken from the bone, processed for standard haematoxylin and eosin staining and evaluated by light microscopy.

Microbiological analysis

Samples from each animal’s sternum were taken aseptically, weighed, homogenized and resuspended in a sterile saline solution. Bacterial counts expressed as S. aureus/g of sternum were determined by serial dilutions of the sample and by colony counting on brain heart infusion agar plates after an overnight incubation.

can allow the efficacy of such treatments to be tested and that can help to develop protection against post-operative mediastinitis and sternal osteomyelitis. There are a number of experimental animal models found in the literature for sternal osteomyelitis or mediastinitis. Only a few of them include MS incision in rats, or employ S. aureus infection as an integral part of the model.

We present here a new experimental rat model that studies the natural history of S. aureus infection following an MS wound involving sternal osteomyelitis and mediastinitis in rats, and prove the effect of a systemic antibacterial as treatment against this infection. Practical employment of the model in evaluating protection against S. aureus osteomyelitis was tested with vancomycin and was found to be suitable for evaluating new antimicrobials and therapy strategies for S. aureus infections. The ultimate aim is to reduce morbidity and mortality rates of infection after cardiac surgery.
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The average concentration of S. aureus in the sternum was calculated from the average of bacterial counts obtained from rats that were sacrificed at each time point. The effect of vancomycin treatment on S. aureus bacterial counts in the sternum was expressed as the median cfu/g of sternal bone for each group.

Statistical analysis

Statistics were run in Stata version 7 (Stata Corp., College Station, TX, USA). The relationship between the size of S. aureus bacterial inoculum injected and bacterial counts from the sternum, during establishment of the model, was quantified using Spearman correlation. The differences in bacterial counts between the vancomycin-treated rat groups and the saline-treated rat groups were analysed using Mann–Whitney’s non-parametric test. Differences were considered to be statistically significant with a P value of 0.05 or less.

Results

Rats included in this study survived MS incision followed by infection with S. aureus using infecting inocula ranging from 10^5 to 10^7 cfu/sternum. Rats receiving an inoculum of 10^6 cfu/sternum overcame the infection completely 10 days after infection, with no bacteria recovered from the sternum. A high infecting inoculum of 10^5 cfu/sternum led to a high mortality rate (four out of five rats). Subjective macroscopic examination 10 days after MS infection revealed an abscess surrounding the sternum with purulent discharge in most of the rats injected with 10^5–10^6 cfu (Figure 1a). Sternal histopathology on day 10 after MS S. aureus infection displayed acute osteomyelitis with bacterial colonies and soft tissue abscesses (Figure 1c), when compared with the control group of rats with MS incision and no bacterial challenge. The latter showed an acute and chronic inflammatory process, with no osteomyelitis, bacterial colonies or soft tissue abscesses (Figure 1b).

To determine the effect of the size of infecting inocula, rats were infected with 1 x 10^5–1 x 10^7 cfu/sternum. Propagation of S. aureus infection in the sternum revealed inoculum-dependent behaviour reflected by sternal bacterial counts and histopathology (Figure 2). There was a direct relationship between the size of the infecting S. aureus inoculum from 1 x 10^3, 1 x 10^6 or 1 x 10^7 and the bacterial counts recovered from the sternum 10 days after infection (3.9 x 10^5, 3.6 x 10^5 and 2.34 x 10^3/g of sternum, respectively, r = 0.8 using Spearman correlation, Figure 2). Histopathology examination of the sternum with 1 x 10^5 cfu of S. aureus per sternum showed scar tissue with chronic inflammatory infiltrates and regenerative changes. A higher inoculum of up to 1 x 10^7 cfu of S. aureus per sternum resulted in acute inflammatory infiltrates with acute sternal osteomyelitis, bone sequestration and soft tissue abscess formation. A challenge inoculum of 1 x 10^7 S. aureus per sternum was chosen for further studies; it was non-lethal; bacteria could be cultured from all the sternum bone preparations and acute osteomyelitis with high bacterial counts was demonstrated in all cases without exception.

Growth kinetics of S. aureus after sternal infection with 10^7 cfu/sternum showed an increase in bacterial counts in the sternum from the second to the eighth day after infection, reaching a peak and then gradually decreasing from day 12 to day 19 post-infection (Figure 3). Vancomycin treatment administered after the MS wound was infected significantly decreased the S. aureus counts in the sternum compared with the S. aureus counts in the placebo (PBS)-treated rats. Median bacterial counts of S. aureus in the sternum bone (lower quartile/upper quartile) were as follows: control group, 1.2 x 10^7 cfu/g (1.5 x 10^5/7.4 x 10^5) and vancomycin group, 4.2 x 10^5 cfu/g (2 x 10^5/2.7 x 10^5) (Figure 4, P = 0.01). No bacteria were recovered from sternum specimens removed from the non-infected MS wound control rats. Sternal histopathology of specimens taken on days 8 and 12 after MS S. aureus infection displayed acute osteomyelitis with bacterial colonies and soft tissue abscesses in the placebo-treated rats. Vancomycin-treated rats showed acute and chronic inflammation with regenerative changes and no bacterial colonies.

Discussion

Post-surgical mediastinitis and sternal osteomyelitis are associated with high morbidity and mortality rates, diminished quality of life and major economic impact. With the expansion of cardiac surgery to include the more severely ill and elderly
from day 8 to day 12.

in bacterial counts recovered from rats injected with the same SA challenge. Error bars represent the SD average bacterial counts recovered from the sternum were determined as

described in the Materials and methods section. Error bars represent the SD in bacterial counts for rats injected with the same SA challenge. The mean average bacterial count recovered from the sternum bone was proportional to the bacterial challenge.

Figure 2. Recovery of *S. aureus* (SA) from the sternum bone 10 days post-sternotomy and infection. Groups of rats (*n* = 5–8) were intra-sternally challenged with different SA inocula (1 × 10^4–1 × 10^7 cfu/sternum). Bacterial counts presented in the figure represent the mean average bacterial counts (normalized/g of bone) recovered from all the sternum specimens, removed from rats injected with the indicated inoculum 10 days post-infection. Error bars represent the SD in bacterial counts for rats injected with the same SA challenge. The mean average bacterial count recovered from the sternum bone was proportional to the bacterial challenge.

Figure 3. Kinetics of *S. aureus* (SA) growth in the sternum during 20 days after median sternotomy followed by SA challenge (10^7 cfu/sternum). Rats (*n* = 4) were sacrificed at different time points post-infection and the mean average bacterial counts recovered from the sternum were determined as described in the Materials and methods section. Error bars represent the SD in bacterial counts recovered from rats injected with the same SA challenge. Maximum bacterial counts in the sternum were obtained from SA infection from day 8 to day 12.

Figure 4. Bacterial counts in the sternum recovered after 12 days of therapy with vancomycin and for the untreated control group. The median bacterial counts recovered from sternum specimens removed from rats (*n* = 12) were determined after median sternotomy incision and infection with 10^7 cfu *S. aureus*/sternum. The boundaries of the box indicate the 25th and 75th percentiles. Vancomycin significantly decreased the *S. aureus* bacterial load in the sternum (*P* = 0.01). Results presented are the averages of two independent experiments.

patients, and the growing prevalence of MRSA in tertiary care and community hospitals, more drug-resistant strains are being isolated from post-surgical MS wound patients.7–13 Clinical studies have shown that post-surgical mediastinitis and sternal osteomyelitis due to MRSA are particularly costly and deadly and are associated with an 11-fold increase in associated mortality and excess hospital charges.7–13

Numerous MS animal models have previously been described in the literature.14,15,17–22 These models were designed to study new surgical techniques and treatment adjuvants, aiming to lower post-operative complications. There are, however, few sternitis or mediastinitis models studied on smaller animals (rodents) that were challenged with bacteria.22–24 To ensure the development of bone infection, some of these models demonstrated osteomyelitis using a foreign body and studied mediastinitis with no MS incision, but instead with direct mediastinal infection.23 Both Ozcan et al.22 and Ogus et al.24 described the effect of adjuvant therapy on an *S. aureus*-infected MS wound rat model, but did not relate their results to *S. aureus* sternal growth kinetics or to sternal histopathology of the infected non-treated group. To the best of our knowledge, no previous article has described the pathophysiology of sternal osteomyelitis and mediastinitis following an MS wound *S. aureus* infection. The most important requirements of a reliable animal model are an infection rate of 100% without spontaneous curing, low mortality of the experimental animals and small variance between the infected animals within a given experiment.

We present this current rat model that studies both bacterial growth kinetics and histopathology of the sternum and challenges it with systemic antibiotics. In our model, the infecting *S. aureus* inoculum was non-lethal, and the bacterial growth from the sternum after infection was found to be inoculum-dependent (Figure 2). The infecting load of 1 × 10^7 cfu of *S. aureus* per sternum resulted in complete recovery from the infection, with no bacterial growth from the sternum. Increasing the bacterial load from 1 × 10^4 to 1 × 10^7 cfu of *S. aureus* per sternum resulted in increasing sternal bacterial growth. Histopathology studies of the sternal samples were consistent with the bacterial counts and displayed acute osteomyelitis and soft tissue abscesses in proportion to the size of the infecting load. The MS incision by itself did not induce sternal infection, in that control rats that underwent MS incision with no infection had no sternal bacterial growth and no signs of osteomyelitis or soft tissue abscesses on histopathology (Figure 1).

An *S. aureus* growth kinetics study demonstrated maximal bacterial growth on days 8–12 after infection (Figure 3). This was when sternal bacterial growth peaked and so emerged as the optimal time to determine bacterial counts.

Challenging *S. aureus*-infected MS wounds with the systemic antibiotic vancomycin significantly decreased sternal bacterial counts (Figure 4). Antibiotic treatment was found to be effective not only in reducing sternal bacterial growth but also by
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attenuating the osteomyelitis process demonstrated by histopathology. Placebo treatment (control rats treated with PBS) had no effect on bacterial growth, with the wounds displaying high bacterial counts and acute osteomyelitis with soft tissue abscesses.

This study has some limitations. The anatomy and collateral blood supply to the sternum in rats may be different from that in humans. Furthermore, this model does not take into account the ischaemic damage to the sternum after harvesting both internal mammary arteries.7,20

There is no consensus on the optimal management of post-sternotomy mediastinitis, but long-term antibiotic treatment is universally accepted as being fundamental to the treatment process.7,10 Although antimicrobial treatment should be initiated promptly, there is no agreement either on the choice of the most suitable drug or on the preference for a combination therapy over monotherapy,10 and new antibacterials are constantly being sought in this era of increasing drug resistance. Our model can provide a framework for studying the efficacy of new antibacterial and treatment modalities in acute mediastinitis and sternal osteomyelitis. Different resistant strains can be challenged with different antibacterials, and various sternal bacterial growths and the histopathology can be studied. This model is simple, relatively inexpensive and reproducible, facilitating comparison of different antibacterial regimens.

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Transparency declarations

None to declare.

Supplementary data

A colour version of Figure 1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References