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What lessons have been learnt from animal models of MRSA in the lung?

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ABSTRACT: ***Staphylococcus aureus*** **is one of the most common causes of nosocomial pneumonia contributing to significant morbidity and mortality. Therapeutic options for patients with methicillin-resistant *S. aureus* (MRSA) infection are limited. In addition, little is known about the *S. aureus* virulence factors that may influence the presentation and prognosis of severe lower respiratory tract infections. Animal models of severe pneumonia allow investigators to control and exclude potential confounders and to examine the influence of comorbid conditions. Therefore, these models may improve our knowledge of the intimate pathophysiological mechanisms affecting pharmacodynamics, pharmacokinetics and efficacy of therapy. So far, animal research studies on MRSA and vancomycin-resistant *S. aureus*, performed both in small and large animal models, have improved knowledge of the mechanisms of disease, which may lead to a better treatment for this severe and complex infection in humans.**

KEYWORDS: **Animal model, lung, methicillin-resistant *Staphylococcus aureus***

Gram-positive bacteria have emerged as an important cause of hospital-acquired and community-acquired infections [1]. *Staphylococcus aureus*, in particular, is one of the most common causes of nosocomial pneumonia contributing to significant morbidity and mortality [2, 3]. An increasing prevalence (from 2% in 1974 to as high as 64% in recent surveys) of methicillin-resistant *S. aureus* (MRSA) among nosocomial isolates has been identified [4–6]. Community-acquired MRSA is also becoming an important public health problem [7]. Although MRSA infection develops mainly in patients with risk factors related to healthcare institutions, it has also been described in the general population [8]. Therapeutic options for patients with MRSA infections are limited. Glycopeptides, such as vancomycin (VCM) and teicoplanin, were the most reliable therapeutic agents against this microorganism. However, since the first report of a Japanese patient harbouring a MRSA strain resistant to VCM in 1996 [9], isolation of reduced VCM susceptibility (SA-RVS) strains has been described in different countries such as the USA, France, Korea, South Africa and Brazil, confirming that the emergence of strains of *S. aureus* with resistance to VCM is a global issue [10]. The design of new therapeutic agents with activity in the face of this microorganism has been developed over recent years. Animal models have contributed significantly to this development and are an essential step between *in vitro* testing and human studies [11]. The availability of an animal model of severe pneumonia where potential confounders are excluded or controlled may improve our knowledge on the intimal mechanisms involving the pathophysiology, pharmacology and efficacy of therapy. Here, we describe lessons that have been learnt regarding MRSA infection using animal models (table 1).

SMALL ANIMAL MODELS OF MRSA-INDUCED PNEUMONIA

There have been few experiments on small animal models of MRSA-induced pneumonia, and they have been mainly carried out using mice and rats because they are cost-effective, easy to manage and genetically malleable. These recent studies have established the use of different routes of *S. aureus* inoculation to induce pneumonia as feasible and reproducible, including the intravenous and intrapulmonary pathways.

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In 1997, SAWAI *et al.* [12] described a novel murine model of acute staphylococcal pneumonia induced by intravenous injection of *S. aureus* enmeshed in agar beads. This animal model is simple and reproducible and resembles blood-borne staphylococcal pneumonia in humans, and it could be useful for investigating the pathogenicity or treatment of staphylococcal pulmonary infection. When *S. aureus* was injected intravenously, the organisms concentrated and remained in the lungs for several weeks. Multiple lung abscesses were evident macroscopically, and histological examination of the infected lung showed multiple lung abscesses around the pulmonary arterioles, consisting of bacterial colonies encircled with fibrin filaments and surrounded by inflammatory cells of neutrophils and macrophages. Using this model, the authors studied the pathogenic role of staphylocoagulase, which is considered to be one of the most reliable determinants for differentiation of this bacterial species from other staphylococci, and also a critical virulence factor. SAWAI *et al.* [12] showed that the titre of staphylocoagulase correlated with the number of viable bacteria isolated from the lung on day 7 after inoculation. By contrast, injection of coagulase-deficient mutant strain was associated with a markedly reduced number of viable bacteria isolated from the lung. These results suggest that staphylocoagulase may play a role in the development of blood-borne staphylococcal pneumonia.

Using this model, the same group of investigators performed a series of studies evaluating the efficacy and pharmacokinetic profile of different agents with activity against MRSA. Their results demonstrated that, compared with VCM and teicoplanin, linezolid clearly reduces bacterial load in the MRSA haematogenous infection model and significantly improves survival rate. Interestingly, these authors reported, for the first time, evidence of *in vivo* efficacy of linezolid against SA-RVS infection. SA-RVS infection was reproduced by pre-treatment of animals with cyclophosphamide and then treating them with the test agent for 10 days using the same doses as those for MRSA. Treatment commenced 1 day after inoculation by intraperitoneal administration of the test agent. As with the MRSA experiments, animals suffered from SA-RVS infection and when treated with linezolid had a better pharmacokinetic profile, lower bacterial counts and a better survival rate than animals with the same infection treated with VCM or teicoplanin [13].

The identification of a MRSA strain resistant to linezolid from a patient with dialysis-associated peritonitis reinforced the need to develop new potent antimicrobial agents against SA-RVS [24]. KANEKO *et al*. [14], using the previously described animal model, evaluated the antibacterial and histopathological effects of DQ-113, a new quinolone, with activity against MRSA and SA-RVS. They compared the new agent with VCM and teicoplanin. DQ-113 significantly reduced the number of viable bacteria in the lung compared with VCM and teicoplanin. Histopathological examination revealed milder inflammatory changes in the group of quinolone-treated mice than in the other groups. The pharmacokinetics parameters, such as the area under the concentration–time curve from 0 to 6 h ($AUC_{0–6}$)/minimum inhibitory concentration (MIC) ratio, or the time that the $AUC_{0–6}$ exceeded the MIC, were the highest for DQ-113. These results suggest that DQ-113 is a potent and effective agent for the treatment of haematogenous pulmonary infections caused by MRSA and SA-RVS strains [14]. Also, this same group of investigators evaluated the effect of quinupristin-dalfopristrin (Q-D) (a complex of streptogramin A and B) and DX-619, a novel des-fluoro(6)-quinolone, with that of VCM against MRSA and SA-RVS. Treatment with Q-D and DX-619 resulted in a significant decrease in the number of viable bacteria in the lungs of mice in a MRSA infection model [15, 16].

Finally, the efficacy of short interfering RNA (siRNA) on the expression of coagulase, one of the most important enzymes in the pathogenesis of MRSA infection, was evaluated by YANAGIHARA *et al.* [17] using the previously described animal model of *i.v.* inoculation of *S. aureus*. They examined the

TABLE 1 Animal models of methicillin-resistant *Staphylococcus aureus* lung infection

Small animal models

Murine model of haematogenous pulmonary infection by intravenous injection of *S. aureus* enmeshed in agar beads

SAWAI *et al.* [12]: animal model validation and role of coagulase (February 1997)

YANAGIHARA *et al.* [13]: efficacy of linezolid against methicillin-resistant or vancomycin-insensitive *S. aureus* (July 2002)

KANEKO *et al.* [14]: effects of DQ-113 against methicillin- and vancomycin-resistant *S. aureus* (August 2003)

YANAGIHARA *et al.* [15]: efficacy of quinpristin-dalfopristin against methicillin-resistant *S. aureus* and vancomycin-insensitive *S. aureus* (November 2004)

YANAGIHARA *et al.* [16]: potency of DX-619 against methicillin-resistant and vancomycin-intermediate *S. aureus* (March 2006)

YANAGIHARA *et al.* [17]: effects of short interfering RNA against methicillin-resistant *S. aureus* coagulase *in vitro* and *in vivo* (December 2005)

Murine model of pulmonary infection by transnasal administration of *S. aureus*

KIMURA *et al.* [18]: animal model validation and factors affecting the course and severity of pneumonia (November 1999)

BUBECK WARDENBURG *et al.* [19]: surface proteins and exotoxins are required for the pathogenesis of *S. aureus* pneumonia (February 2007)

BUBECK WARDENBURG *et al.* [20]: vaccine protection against *S. aureus* pneumonia (February 2008)

Murine model of pulmonary infection by instillation into the lung of *S. aureus* in ventilated animals

MCELROY *et al.* [21]: alpha-toxin damages the air–blood barrier (October 1999)

Large animal models

Ovine ventilated model of pulmonary infection by instillation by bronchoscope of *S. aureus*

ENKHBAATAR *et al.* [22]: animal model validation (May 2008)

Piglet ventilated model of pneumonia induced by instillation by broncoscope of *S. aureus*

SIBILA *et al.* [23]: animal model validation (2007)

inhibitory effect of siRNA on staphylocoagulase *in vitro* and *in vivo*. The results showed that siRNA inhibited both mRNA expression and the activity of MRSA coagulase *in vitro*. The *in vivo* results revealed that the siRNA was effective in reducing the bacterial load. Targeting coagulase with siRNA appears to be a novel and promising strategy for treating MRSA infections.

Regarding the studies on acute staphylococcal pneumonia induced by intranasal or intrapulmonary injection of *S. aureus*, in 1999 KIMURA *et al.* [18] examined several factors that may affect the course and severity of *S. aureus* pneumonia in five strains of immunocompromised mice. Immunosuppression was induced by injection of cyclophosphamide and impairment of mucociliary clearance by intranasal instillation of formalin. Authors found that pre-treatment with formalin plus cyclophosphamide was associated with a significant increase in lung bacterial counts and a high mortality. Moreover, CBA/J mice represented the most susceptible strain among those examined. Therefore, they demonstrated that neutropenia and impaired mucociliary clearance were major factors that influence the severity of pneumonia in mice. However, although this animal model emphasised the inflammatory response to intrapulmonary *S. aureus*, characterisation of bacterial virulence factors was not possible because of the quick clearance of *S. aureus* from the lungs. In contrast, BUBECK WARDENBURG *et al.* [19] more recently developed a transnasal murine model of *S. aureus* pneumonia in adult and immunocompetent C57BL/6J mice. They found that this model closely mimics the clinical and pathological features of pneumonia in human patients and allows investigation of virulence factors such as the pore-forming β-barrel toxin Hla. Later, these authors used this experimental model to examine Hla as a target for the development of vaccines that combat *S. aureus* lung infection [20]. They demonstrated that the relative level of Hla expression by distinct *S. aureus* strains correlated with the virulence properties of the organism. Moreover, they reported that active immunisation with a mutant form of Hla protected mice from staphylococcal pneumonia, and that this protection correlated with reduced microbiological and pathological evidence of disease. In addition, these authors passively immunised animals with Hla-specific antibodies and demonstrated that passive immunoprotection protected animals from *S. aureus* pneumonia, correlating with favourable alterations in the cytokine profile of the host. In agreement with these results, MCELROY *et al.* [21] determined the role of alpha-toxin by developing a rat model of *S. aureus*-induced pneumonia. These authors instilled *S. aureus* strain 8325-4 into the lungs of ventilated Sprague–Dawley rats and demonstrated that the function of the air–blood barrier was impaired in *S. aureus*-induced pneumonia and that alpha-toxin was an important cause of damage to the air–blood barrier.

Recently, a study by REYES *et al.* [25] used a similar murine pneumonia model to compare the efficacy of telavancin with that of VCM and linezolid against MRSA. In particular, the authors inoculated 10^7 CFU of MRSA onto the tip of the nares of neutropenic mice, and 12 h and 24 h after inoculation the animals were dosed with telavancin, VCM or linezolid. They found that when dosed both at 12 h and at 24 h after inoculation, animals treated with telavancin had a significantly greater reduction in lung bacterial titre than those treated with VCM or linezolid. Moreover, when the animals were dosed at 24 h after inoculation, telavancin and VCM-treated groups showed significantly greater improvement in survival of mice than did controls and linezolid-treated groups, suggesting the potential utility of telavancin for treatment of MRSA pneumonia.

LARGE ANIMAL MODELS OF MRSA-INDUCED PNEUMONIA

Although small animals offer the advantages of low cost, genetic malleability and high throughput, they cannot reproduce many aspects of human pulmonary pathophysiology. For this reason, it is very important to use physiologically relevant large animal models whenever possible. However, if there have been few experimental studies on the development of small animal models of MRSA-induced pneumonia, there have been even fewer using large animal models of MRSA. Nevertheless, it is important to point out the relevance of the study of ENKHBAATAR *et al.* [22], using an ovine model of MRSA-induced pneumonia and sepsis that closely mimics hyperdynamic human sepsis. Authors established a standardised and reproducible model of MRSA-induced septic pneumonia by instillation of *S. aureus*, with and without smoke injury induced by inhalation of cotton smoke. They found that animals exposed to smoke inhalation and MRSA showed the signs of severe sepsis-related multiple organ failure 3 h after insult. Pulmonary dysfunction was characterised by deteriorated gas exchange and increased ventilatory pressure. Moreover, animals showed significantly greater lung tissue water, myeloperoxidase activity and cytokine production compared with uninjured animals. Conversely, using this novel ovine model, the authors found that all these changes were accompanied by an increase in plasma nitrite/nitrate and reactive nitrogen species in the lungs, suggesting that excessive nitric oxide production may be involved in the pathogenic process.

Another relevant study about the development of a large animal model of MRSA-induced pneumonia was carried out in pigs. SIBILA *et al.* [23] validated a porcine model of MRSA pneumonia and studied the time-course of biological markers and histopathological changes. The authors found that, in ventilated pigs, bronchoscopic inoculation of MRSA induced pneumonia at 12 h and severe pneumonia at 24 h. This severity was associated with an increase in interleukin (IL)-6, IL-8 and tumour necrosis factor-α at 24 h after inoculation. Therefore, the authors showed that this experimental model in ventilated piglets closely mimics MRSA human pneumonia and it could lead to future studies on the effects of different antimicrobial therapies against MRSA pneumonia.

CONCLUSION

S. aureus is one of the most common causes of nosocomial pneumonia contributing to significant morbidity and mortality. Therapeutic options for patients with MRSA infection are limited. In addition, little is known about the *S. aureus* virulence factors that may influence the presentation and prognosis of severe lower respiratory tract infections. Animal models of severe pneumonia allow investigators to control and exclude potential confounders and to examine the influence of co-morbid conditions. Therefore, these models may improve our knowledge on the intimate pathophysiological mechanisms affecting pharmacodynamics, pharmacokinetics and efficacy of therapy. So far, animal research studies on MRSA

and SA-RVS, performed both in small and large animal models, have provided further insight into the mechanisms of disease that may lead to a better treatment of this severe and complex infections in the human.

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STATEMENT OF INTEREST

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