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Preclinical Models of Brucellar Spondylodiscitis

Xiaoyu Cai, Tao Xu, Maierdan Maimaiti and Liang Gao

Abstract

Brucellar spondylodiscitis, the most prevalent and significant osteoarticular presentation of human Brucellosis, is difficult to diagnose and usually yields irreversible neurologic deficits and spinal deformities. Relevant aspects of *Brucella* pathogenesis have been intensively investigated in preclinical models. Mice, rats, rabbits, and sheep are representing available models to induce Brucellosis. Evaluation of Brucellar spondylodiscitis may be performed using a large variety of methods, including plain radiography, computed tomography, magnetic resonance imaging, histological analysis, blood test, and bacteria culture. This chapter focuses on these preclinical models of Brucellar spondylodiscitis. The requirements for preclinical models of Brucellar spondylodiscitis, pearls and pitfalls of the preclinical model establishment, and comprehensive analyses of Brucellar spondylodiscitis in animals are also depicted.

Keywords: Animal models, Preclinical models, Brucellar spondylodiscitis, Brucellosis

1. Introduction

Brucellosis, an infectious disease caused by the *Brucella* bacteria in both humans and animals, leads to a significant impact on the public health and the animal industry [1]. Since 1950, comprehensive measures against Brucellosis were undertaken in China and a great number of achievements had been made in its prevention and control [2]. It, however, remains a serious public health issue, and much remains to be accomplished to reach the goal of controlling human and animal Brucellosis in China. As the most common and significant osteoarticular presentation of human Brucellosis, Brucellar spondylodiscitis has a variable course with a long latency between the onset of symptoms and the radiologic changes' appearance with and unspecific clinical symptoms, hindering an early intervention to prevent irreversible neurologic deficits and spinal deformities (**Figure 1**) [4, 5]. It is an endemic disease in areas of sheep farming, which is also widespread among farmers, animal breeders, veterinarians, and veterinary technicians as an occupational disease [6, 7]. Due to the late-onset radiological findings, slow growth rate in blood cultures, and complexity of the serodiagnosis, timely and accurate diagnosis of Brucellar spondylodiscitis is still a challenge for clinicians [8, 9].

Preclinical models exhibiting symptoms comparable to those in humans are essential for the translation of preclinical findings into clinical practice [10–13]. Relevant aspects of the *Brucella* pathogenesis have been intensively investigated in both *in vitro* and preclinical *in vivo* models. Several preclinical models are available to mimic Brucellar spondylodiscitis and provide beneficial platforms allowing the

Figure 1.

A typical case of noncontiguous multiple-level Brucellar spondylodiscitis with an epidural abscess in a 57-year-old man with a 6-month history of low back pain, restricted range of motion, fever, chills, and night sweating. (A) the midsagittal magnetic resonance imaging revealed increased signal intensity (arrows) involving the T2-T3, T8-9, T11-12, and L4-5 disks and vertebral bodies. (B) Pathologic signal changes were identified, compatible with a 14 × 8-mm paraspinous abscess (L5), with low signal intensity on T1-weighted images, high signal intensity on T2-weighted images, and post-contrast peripheral enhancement (arrow) [3].

management and exploration of translational investigations of medical device and novel therapeutics [14–16].

This chapter focuses on these preclinical models of Brucellar spondylodiscitis. The requirements for preclinical models of Brucellar spondylodiscitis, pearls and pitfalls of the preclinical model establishment, and comprehensive analyses of Brucellar spondylodiscitis in animal models are also deliberated.

2. Requirements for preclinical models of Brucellar spondylodiscitis

Investigators have previously established Brucellosis models in many diverse animals [15], however the animal candidates for stimulating *in vivo* Brucellar spondylodiscitis should be suitable for both the implantation of the *Brucella* bacteria and the convenience for the anatomical morphology research (**Table 1**). Common requirements for preclinical models of Brucellar spondylodiscitis are diverse, including but not limited to:

1. The geometrical size and anatomical structure of the animal spine should be comparable to the human spine.
2. The biomechanical properties of the animal spine should be close to the human spine.

Species	Characteristics of spine	References
Mouse	Significantly smaller size than the human spine Different mechanical loading from the human spine Advantages of easy surgical manipulations	[17, 18]
Rat	Normalized disc height in rats higher than that in humans Vertebral dimensions varying more in rats than in humans Vertebrae slenderer in rats than in humans	[18, 19]
Rabbit	Seven lumbar vertebral segments in rabbit and the lowest segment connected to the sacrum (similar to humans) Biomechanical behavior of the lumbar spine comparable with the human lumbar spine	[18, 20, 21]
Sheep	Spine size larger than humans (particularly in vertebral body height and pedicle height) Similar increasing trend of spinal canal width and depth to humans	[22]

Table 1.
Comparison of the spine structure of animal models of brucellosis.

3. The model needs reflect the clinical nature of the Brucellar spondylodiscitis.
4. The radiographic characteristics of Brucellar spondylodiscitis in specific animals should be analogous to the human setting.
5. The generated preclinical data can be translated into the clinical situation and further benefit the clinical treatments.

3. Preclinical models of Brucellar spondylodiscitis

Mice, rats, rabbits, and sheep represent the available candidates to induce Brucellosis [23–26]. However, the preclinical model of Brucellar spondylodiscitis with a possible high translational efficiency has only been established in rabbits so far [14].

3.1 Mice

Mice are the most extensively used to investigate chronic infections of *Brucella* [15]. Enright and colleagues constructed the Brucellosis model with the mouse by intravenous injection of 5×10^4 colony forming units (CFU) of either *Brucella* attenuated strain 19 or *Brucella abortus* strain 2308 to confirm *Brucella abortus* produces a chronic granulomatous response in mice, and extend earlier studies in demonstrating that prominent acute and subacute inflammatory responses also occur [27]. Steven *et al.* made the mouse model with the method of intraperitoneal injections of 10^5 CFU of *Brucella* strain 2308 or 10^7 CFU of either strain 19 or RB51 [28]. Tobias’ Brucellosis mouse model was built by intraperitoneally infected with 10^6 CFU of *Brucella abortus* strain 2308 [29]. The spleen is the most heavily colonized organ with voluminous histiocytic infiltrates and multifocal microgranulomas [27–29]. Besides the spleen, the liver is also a common site for colonization and replication of *Brucella* in the mice [27, 30, 31]. Mice infected with virulent strains of *Brucella* have mild to moderate hepatitis characterized by neutrophilic infiltrate at early stages of the infection, followed by histiocytic infiltrate with epithelioid cells and microgranulomas at its chronic stages [27, 29]. It is noteworthy that *Brucella* infection in mice results in lesions that mimic those described in chronic infections

in humans, and patients with chronic Brucellosis may develop splenomegaly and hepatomegaly [32]. In addition, multifocal granulomas with epithelioid macrophages are also detected in the liver or spleen parenchyma of patients who were infected with *Brucella* [33, 34]. Chronic infection of the *Brucella* in humans may also yield osteoarticular alterations, including osteoarthritis and spondylodiscitis [35]. Rajashekara *et al.* reported that mice may develop bacterial colonization in periarticular tissues during the chronic stages of *Brucella melitensis* infection [36]. In mice surviving over 45 days after intraperitoneal infection of *Brucella melitensis*, the bioluminescent *Brucella melitensis* were detected in the vertebral joints of their tails, suggesting that the mice might be a useful model for the study of human osteoarticular diseases, including the Brucellar spondylodiscitis [16]. Comparisons of disease manifestations (e.g. timing of the disease onset, structural alterations of the spines, and definite localization of the bacterial foci) between animal and human Brucellar spondylodiscitis would be beneficial for evaluating its potential translational values and further recognizing specific histological, radiographic, and clinical signs, allowing for an early detection and intervention of human Brucellar spondylodiscitis.

The mouse model has been utilized for the evaluation of the efficiency of different pharmacotherapies for human Brucellosis [37–39]. Several lines of evidence suggest that mice treated with ciprofloxacin, by subcutaneous (40 mg/kg), digestive (200 mg/kg), or intraperitoneal (20 mg/kg) route, are not able to control the infection of *Brucella melitensis*. In contrast, mice treated with doxycycline (40 mg/kg) at 24 hours after infection efficiently clear the infection [38, 39]. Shasha *et al.* showed that mice administered intraperitoneally with doxycycline (40 mg/kg/day) or rifampin (25 mg/kg/day) had high levels of antibiotics in the blood following 1-hour postinjection (doxycycline: 5.4 µg/ml and rifampin: 18 µg/ml, respectively) and were able to clear the infection. The treatment regimen of the usage of doxycycline and rifampin is consistent with the therapeutic protocols of human Brucellosis.

3.2 Rats

The rats, more resistant to *Brucella* infection than the mice [15], can develop the persistent bacteremia, and do not have a spontaneous cure over the 1-month infection [23]. Therefore, rats can serve as a model candidate to evaluate the increased susceptibility to *Brucella* infection, which mimics the chronic symptoms in patients. Yumuk *et al.* analyzed the rat model by intraperitoneal injection of 2×10^5 to 4×10^5 CFU of *Brucella melitensis* strain 16 M [23]. Moreover, the rat model has also been used to analyze the efficacy of various antibiotics to treat Brucellosis [40–42] and to evaluate the pathological properties and clinical characters of *Brucella* infection during the pregnancy [43]. Also, the similar morphology of the spine at the axial plane between rats and humans supports their application as an applicable candidate for the spine research [19, 44]. Since patients with Brucellar spondylodiscitis are commonly detected at the late phase of the disease, rats, as the potential model of chronic Brucellar spondylodiscitis, may be beneficial to assist to examine specific remedies against those chronic manifestations within a clinically relevant setting.

The usefulness of pharmacotherapy has been investigated in the treatment for Brucellosis in the rat model. Geyik *et al.* reported that rats were administered orally with rifampicin (50 mg/kg/day) and doxycycline (40 mg/kg/day), or spiramycin (50 mg/kg/day), or a combination of spiramycin and rifampicin at the same dose for 21 days [40]. All the rats were cured with the treatment results that the effectiveness of spiramycin and rifampicin plus spiramycin were similar to rifampicin plus

doxycycline. This result is helpful for the effective alternative in the treatment of human Brucellosis. Furthermore, Sezak and colleagues proposed that moxifloxacin might be an alternative choice in the treatment of Brucellosis [42]. Doxycycline (10 mg/kg/day) plus rifampicin (6 mg/kg/day) were administered intragastrically in Yumuk's study [41]. In the experiment group, all the rats received a liquid diet containing ethanol. The cure rate was 64.71% in ethanol-fed and 100% in ethanol free group. The results suggest that ethanol ingestion diminishes the efficacy of doxycycline plus rifampicin combination therapy of rat Brucellosis model, which holds implications for the treatment programme for human Brucellosis.

3.3 Rabbits

Compared with other animals, rabbits are medium-sized animals which frequently used in spine research with various advantages [45]. The rabbit spine maintains considerable morphological and structural similarities to the human spine, and its body size allows for an adequate exposure during the surgical interventions [46, 47]. Furthermore, rabbits yield higher possibilities than rodents to successfully translate preclinical discoveries into humans [48, 49]. Similarly, compared with larger animals, radiographic analyses are largely convenient in rabbits particularly for *in vivo* explorations [50].

Age is a critical issue to be considered when establishing a rabbit model of local restricted Brucellar spondylodiscitis. The significant variance of the innate immune response between young and adult rabbits against infections of foreign microbes should be recognized [14]. Virus related studies highlighted the significantly superior innate immune system in young rabbits (<4 weeks) over adult rabbits, which contributed to their distinct susceptibility to virus infections [51–53]. These data may partially explain the fact that adult rabbits are only partially susceptible to *Brucella* infection [15], and about 20% of infected animals developed a very short and sporadic bacteremia [25]. In contrast, our previous experiment within young rabbits showed that 83.4% (10 out of 12) rabbits were successfully infected by the intraosseous injection of 3×10^7 CFU of *Brucella melitensis* strain M5–90 [14]. Moreover, to mimic a local restricted inflammation without systemic dissemination of the microbes, studies via a local injection intervention with a reduced dose within young rabbits can avoid the unanticipated animal death due to the local or systematic administration with a relatively larger dosage within adult rabbits. Therefore, even with a relatively smaller size of the spine than adult rabbits, young rabbits may be more suitable to establish such a local restricted model of Brucellar spondylodiscitis.

Of note, despite the animal model for Brucellar spondylodiscitis has been established in rabbits, no studies about its treatments are available for the rabbit Brucellosis.

3.4 Sheep

Although *Brucella ovis* is one of the few classical *Brucella* species that do not have zoonotic potential, this organism is considered a major cause of reproductive failure in sheep [54]. The attenuated vaccine strain *Brucella melitensis* Rev.1, against *Brucella* infection of sheep and goats, are still the most efficient ones available among living vaccines [55]. When the bacteria are administered by the classic subcutaneous method ($10^9 - 2 \times 10^9$ CFU) in the sheep, a long-lasting serologic response is subsequently yielded. Primary manifestations of *Brucella ovis* infection in sheep are lesions of the epididymis and testis in males (e.g., epididymitis and orchitis), placentitis and abortion in ewes, and occasionally perinatal death

in lambs [56], as well as arthritis [57]. The sheep spine is relatively larger than humans, particularly in vertebral body width, which would be advantageous for the easier surgical operation [22]. However, sheep are expensive to house and the operation requires special settings, which also hinder the widely accessibility of this model [58].

Oxytetracycline combined with streptomycin were evaluated for eliminating *Brucella melitensis* from naturally infected sheep. The following treatment regimens were equally effective in eliminating *Brucella* in the sheep: oxytetracycline (20 mg/kg/day) intravenously daily for 6 weeks combined with streptomycin (20 mg/kg/day) intramuscularly for 3 weeks; long-acting oxytetracycline 20 mg/kg intramuscularly every 3 days for 6 weeks plus streptomycin 20 mg/kg intramuscularly every 3 days for 3 weeks; long-acting oxytetracycline mg/kg intramuscularly every 3 days for 6 weeks combined with streptomycin 20 mg/kg intramuscularly every 3 days for 3 weeks [59]. The data indicated the most effective and practical regimen for eliminating *Brucella* in the sheep.

4. Pearls and pitfalls of the preclinical model establishment

The key to establish the preclinical model is to implant the *Brucella* into the superior zone of the anterior column of lumbar vertebral body (Figure 2). In rabbits, to boost a localized inoculation into the vertebral body and minimize the vascular dissemination, the *Brucella* bacteria should be meticulously implanted within the superior zone of the anterior column of the L6 vertebral body of rabbits,

Figure 2. Schematic of the experimental challenge injection with *Brucella melitensis* into the 6th lumbar vertebrae (L6) of rabbits [14].

where the direct vertebral body feeding capillaries scantily supplied [14]. During the procedure, the insertion site, direction, and drill depth of the Kirschner wire should be properly monitored. The open surgery is recommended to expose the target vertebrae for the intraosseous injection rather than the radiograph-guided percutaneous injection to ensure an accurate localization of the bacterial inoculation. Also, to avoid iatrogenic nerve injuries intraoperatively, the different patterns of the level of the nerve root origin and adjacent vertebra in animals from humans need to be recognized.

5. Comprehensive analyses of Brucellar spondylodiscitis in preclinical models

Several techniques can be applied for the evaluation of Brucellar spondylodiscitis in animals. To observe the targeted vertebral body and intervertebral disc

Classification	MRI characteristics
Discitis	Regional inflammation involving intervertebral disc Disc space narrowing Low signal on T1-weighted image mixing high signal on T2-weighted image
Spondylitis	Regional inflammation involving adjacent vertebrae Vertebrae diffuse marrow edema Homogeneous or uneven low signal on T1-weighted image of vertebrae
Paraspinal/ psoas abscess	Regional inflammation involving paraspinal or psoas Paravertebral abscess Psoas abscess
Appendicitis	Regional inflammation involving appendicitis Low signal on T1-weighted image High signal on T2-weighted image
Compound	Endemic inflammation involving two or more parts of vertebral and paravertebral structures T1-weighted image reveals incomplete heterogeneous hypointensity T2-weighted image reveals hyperintensity

Table 2.
Magnetic resonance imaging (MRI) classification of Brucellar spondylodiscitis adapted from [14].

Figure 3.
Histological descriptions of the paravertebral soft tissue of Brucellar spondylodiscitis model in rabbits [14]. Hematoxylin and eosin staining features predominant lymphocyte and monocytes infiltration with sparsely distributed epithelioid cells and multinucleated giant cells. Yellow arrows indicate multinucleated giant cells and red arrows specify epithelioid cells. Yellow arrowheads define lymphocytes and red arrowheads display monocytes (magnification: (left) × 40; (right) × 100).

postoperatively, *in vivo* plain radiography, computed tomography, and magnetic resonance imaging (MRI) analyses should be performed under a general anesthesia. Specifically, the MRI findings were classified into five types, such as discitis type, spondylitis type, paraspinal/psoas abscess type, appendicitis type, and compound type, with a previously reported classification system (**Table 2**) [14]. Histological analysis for samples biopsied from the affected intervertebral disc, upper and lower end-plates, paravertebral soft tissue, psoas, and granulation tissue is highly recommended for identify the pathological pattern of the infection [60]. The pathological characteristics of Brucellar spondylodiscitis in rabbits are the massive inflammatory cell infiltration without evident bony erosions within the biopsied paravertebral structures, including lymphocytes, monocytes, and multinucleated giant cells (**Figure 3**) [61]. Blood test and bacteria culture can be done to further investigate the pathophysiological status of Brucellar spondylodiscitis.

6. Preclinical evaluation of therapeutic interventions and vaccines

Pharmacotherapy is the main therapeutic intervention for the treatment of human Brucellosis, including Brucellar spondylodiscitis. Ciprofloxacin, doxycycline, rifampin has been utilized for the evaluation of the efficiency of different pharmacotherapies for human Brucellosis [37–39]. Different combination of rifampicin, doxycycline, and spiramycin or moxifloxacin were analyzed in rat Brucellosis model for the potential therapeutic options [40, 42]. Oxytetracycline combined with streptomycin were evaluated in the sheep for the test of practical and cost-effective treatment regimen for Brucellosis [59]. Additionally, as a new antibiotic carrier, the microspheres have been used to test for a treatment effect of *Brucella* infection [62]. Microspheres based on poly (lactide-co-glycolide) wherein gentamicin entrapped have been tested to target gentamicin to the cells of the monocyte–macrophage system to reduce drug toxicity and control its release over several days [63]. However, Prior and colleagues reported that mice infected with virulent strains of *Brucella* and treated with three intraperitoneal doses of 100 µg gentamicin microspheres were not able to reduce bacterial load in the spleen after 1 and 3 weeks posttreatment [37].

Surgery should be performed to treat Brucellar spondylodiscitis if the pharmacotherapy is poorly done. The indications for surgery included the following: persistent pain due to spinal instability, severe or progressive neurologic dysfunction due to nerve root compression by inflammatory granuloma or epidural abscesses, and no response to antibiotic therapy [9, 64]. The preclinical model is critical for the research of the improvement of surgery protocols. However, the rabbit model was originally developed for the study of Brucellar spondylodiscitis. Future studies are needed to further refine the surgical procedures.

By far, the quality of live vaccines that are commercially used for preventing animal Brucellosis is evaluated in mouse models. Live *Brucella abortus* (strain S19), which is the most widely used vaccine in cattle, has been tested in mice. The mice were previously treated with 10^5 CFU of *Brucella* reference strain S19 vaccine [15]. All mice were injected intraperitoneally with 10^5 CFU of *Brucella* 30 days after the vaccination. The commercial vaccine is considered efficient when mice have a significantly lower bacterial load than the unvaccinated control group and when the vaccinated group has similar immunogenicity value to the mice group vaccinated with S19 reference strain [15]. Recently, a new candidate vector vaccine against human Brucellosis based on recombinant influenza viral vectors subtypes H5N1 expressing *Brucella* outer membrane protein (Omp) 16, L7/L12, Omp19 or copper/zinc superoxide dismutase proteins has been developed [65]. The effectiveness of

the new anti-Brucellosis vector vaccine was determined by studying its protective effect after conjunctival, intranasal and sublingual administration in doses 10^5 50% egg infective dose (EID₅₀), 10^6 EID₅₀ and 10^7 EID₅₀ during prime and boost vaccinations of animals, followed by challenge with virulent strains of *Brucella* infection. Double intranasal immunization of guinea pigs at a dose of 10^6 EID₅₀, which provided 80% protection of guinea pigs from *Brucella* infection [65]. The proposed vaccine has achieved the best level of protection, which in turn provides a basis for its further promotion.

7. Challenges and outlooks

During the past decades, significant progresses to diagnose and treat the Brucellar spondylodiscitis have been achieved [66–69]. However, many obstacles still exist to be overcome in order to employ and utilize new strategies to refine early detection, diagnosis, therapy [70, 71]. Regarding the basic research, no appropriate vaccines exist due to an incomplete understanding of the mechanisms of human Brucellosis, including Brucellar spondylodiscitis. Clinically, the early and differential diagnosis of the Brucellar spondylodiscitis is challenging, especially in the early phases of the disease. Also, pharmacotherapy is the main clinical therapeutic modality for Brucellar spondylodiscitis and should be individually tailored; however, medication selection, administration, dosage, and duration are still largely debatable.

The ideal preclinical models should reflect the precise clinical characteristics of the human Brucellar spondylodiscitis and serve as a platform to explore the potential vaccines, examine novel diagnostic methods, and preselect innovative therapeutics [72–74]. More investigations in the future are still required to determine the optimal clinically relevant large preclinical model, to identify the efficacy-associated factors (e.g. age, joint size, gender, and dosage), to compare possible dissimilarities between models with local contained lesions or systematic spreading.

8. Conclusions

The pathogenesis, diagnosis, and treatment approach of Brucellar spondylodiscitis has recently become a clinical and research focus. Brucellar spondylodiscitis with highly variable clinical manifestations are practically challenging to be mimicked with laboratory preclinical models. More human-relevant preclinical models should be established to provide better insights into the sophisticated mechanism of human Brucellosis and early interventions of Brucellar spondylodiscitis.

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