Cytotherapy, 2014; 16: 56–63

International Society for Cellular Therapy
ISCT®

Marrow mesenchymal stromal cells reduce methicillin-resistant *Staphylococcus aureus* infection in rat models

YUAN YUAN[1,*], SONGYI LIN[1,*], NA GUO[1], CHENGCHENG ZHAO[2], SUXIA SHEN[1], XIUJUAN BU[1] & HAIQING YE[1]

[1]*Department of Food Quality and Safety, College of Agriculture, Jilin University, Changchun, China, and* [2]*College of Animal Science and Veterinary Medicine, Jilin University, Changchun, China*

Abstract

Background aims. Staphylococci account for a large proportion of hospital-acquired infections, especially among patients with indwelling devices. These infections are often caused by biofilm-producing strains, which are difficult to eradicate and may eventually cause bacteremia and metastatic infections. Recent evidence suggests that mesenchymal stem cells can enhance bacterial clearance *in vivo*. *Methods.* In this study, a rat model with carboxymethyl cellulose pouch infection was used to analyze the efficacy of bone marrow–derived mesenchymal stromal cells (BMSCs) against the methicillin-resistant *Staphylococcus aureus*. *Results.* The results showed that the administration of BMSCs effectively reduced the number of bacterial colonies and the expression of many cytokines and chemokines (such as interleukin [IL]-6, IL-1β, IL-10 and CCL5). Unlike the fibroblast control groups, the pouch tissues from the BMSC-treated rats showed the formation of granulations, suggesting that the healing of the wound was in progress. *Conclusions.* The results indicate that the treatment of BMSCs can reduce methicillin-resistant *S aureus* infection *in vivo*, thereby reducing the inflammatory response.

Key Words: *mesenchymal stromal cells, methicillin-resistant Staphylococcus aureus, rat models*

Introduction

Staphylococci account for a large proportion of hospital-acquired infections, especially among patients with indwelling devices (1). These infections are often caused by biofilm-producing strains, which are difficult to eradicate and may cause bacteremia and metastatic infections (2). In the tissues that were removed from patients with recurrent *Staphylococcus aureus* infections, the cells are frequently organized in confluent colonies, showing a biofilm-like appearance. Once a biofilm has been established, it is a major concern for clinicians in the treatment of infectious disease because of its resistance to a wide range of antibiotics. Methicillin-resistant *S aureus* (MRSA) is resistant not only to β-lactams, such as methicillin, but also to most other antimicrobial agents (3). Vancomycin has been used to treat MRSA infection (3,4). However, the increasing prevalence of multidrug-resistant strains, and more recently, the appearance of strains resistant to vancomycin, raises the specter of untreatable staphylococcal infections (5). Therefore, in addition to antibiotic chemotherapy, a new strategy to treat MRSA infection is needed.

Mesenchymal stromal cells (MSCs) or marrow stromal cells (6) are a type of non-hematopoietic, adult stem-like cells that can be isolated from bone marrow and readily expanded *in vitro* (7,8). MSCs are plastic-adherent, fibroblast-like, multipotent non-hematopoietic progenitor cells (9) that can differentiate into at least three cell lineages: osteogenic, adipogenic and chondrogenic cells. In addition, MSCs can differentiate into various tissues and cell types of mesenchymal and non-mesenchymal origin, including tenocytes, skeletal myocytes, neurons and cells of the visceral mesoderm (10–12). Bone marrow–derived stem or progenitor cells have received increasing interest for the treatment of diseases associated with inflammation and organ injury (13–17). A variety of studies showed that MSCs share functions with innate immune cells, such as the secretion of cytokines and immunomodulatory mediators, chemokine-regulated migration and the expression of surface molecules for interaction with

*These authors contributed equally to this work.

Correspondence: **Haiqing Ye**, MD, Department of Food Quality and Safety, College of Agriculture, Jilin University, Xi'an Road 5333, Changchun 130062, People's Republic of China. E-mail: yehq@jlu.edu.cn

(Received 22 January 2013; accepted 8 June 2013)

http://dx.doi.org/10.1016/j.jcyt.2013.06.002

various leukocyte subsets (18). Recent evidence suggests that MSCs can also enhance bacterial clearance *in vivo* (19,20). On the basis of the reported research, we hypothesized that MSCs would exert a beneficial effect in eradicating MRSA.

Many attempts have been made to generate experimental bacterial infection models that reproduce those formed in the human body to attempt the effective eradication of bacteria. Of these, the carboxymethyl cellulose (CMC) pouch infection model in rats has been used to test the efficacy of anti-microbial agents (21). The rat pouch model allows the measurement of the anti-microbial effect of the agent penetrating through the bacterial biofilm layer. With the use of this system, we estimated the efficacy of therapy with bone marrow mesenchymal stromal cells (BMSCs) against the lesions induced by implanted MRSA.

Methods

BMSC isolation, purification and identification

Isolation and culture of BMSCs was performed by a previously described method with some modifications (22). Newborn Wistar rats were killed by cervical dislocation and were then put into 750 mL/L alcohol for 15 min. Two femurs were dissected out from one rat under sterile conditions. Muscles on the femurs were stripped cleanly without leaving any tissue. Direct adherence and density gradient centrifugation methods were used in the purification of BMSCs, and routine and modicum medium change methods were used in culturing the BMSCs. Medullary contents of femurs were washed with low-glucose Dulbecco's modified Eagle's medium (DMEM-LG), and the cells obtained were layered over an equal volume of Percoll solution (1.073 g/mL). Mononuclear cells (MNCs) were recovered from the gradient interface and washed with phosphate-buffered saline after centrifugation at 1500 rpm for 10 min. The MNCs were suspended in 5 mL of DMEM-LG, which was supplemented with 15% fetal bovine serum, and finally, the MNCs were cultured in 25 cm^2 of plastic cell culture flasks at a density of 10^6/mL. The cells were maintained in a humidified incubator at 37°C in 5% CO_2. In the first 3 days, the entire culture medium was replaced every day, and thereafter, only half of the culture medium was replaced every third or fourth day. When the cells reached 70–80% confluence, the cells were passaged after dislodging them from the growth surface by use of 0.25% trypsin.

BMSCs that were grown to five generations were used in all *in vivo* experiments. Cell morphology was observed by use of Giemsa staining and compared with the fibroblast. The expression of surface markers CD14, CD34, CD105, CD73 and CD90 was determined by use of flow cytometry (BD FACSCalibur, USA). The differentiation potential of purified BMSCs was evaluated by induction with ossification inducer and adipogenic inducer (22,23). Ossification inducer consisted of DMEM-LG supplemented with 0.1 mmol/L dexamethasone, 10 mmol/L β-glycerol phosphate and 0.2 mmol/L ascorbic acid (Sigma, St Louis, MO, USA), and osteogenesis was assessed by use of chinalizarin staining. Adipogenic medium consisted of DMEM high-glucose medium (DMEM-HG) supplemented with 0.5 mmol/L 3-isobutyl-1-methylxanthine, 1 μmol/L hydrocortisone, 0.1 mmol/L indomethacin and 10% rabbit serum (Sigma), and adipogenesis was assessed by use of oil red O staining.

Bacterial strains and culture conditions

MRSA strain W3275 was isolated from clinical specimens obtained from patients at the First Hospital of Jilin University. The isolated strains were confirmed as *S aureus* by polymerase chain reaction (PCR) amplification of the 16S ribosomal RNA and the coagulase test. It was identified as an MRSA strain on the basis of colony formation on selective salt agar plates containing 6 mg/mL oxacillin. W3275 strain was pre-cultured in trypticase soy broth (Qingdao Hope Bio-Technology Co Ltd, Qingdao, China) at 37°C for 20 h before use.

Mouse biofilm infection model and administration of BMSCs

Female Wistar rats weighing 180–200 g (8–12 weeks old) were purchased from the Experimental Animal Center of Jilin University. The local infection model, with CMC placed in the dorsal region of rats, was used in this study and has been described previously (21,24). Briefly, air pockets were produced by subcutaneous injection of 10 mL of sterile air into the intra-scapular area of the back with the use of a 21-gauge needle after hair was trimmed off with a hair clipper. The next day (recorded as day 1), the pouch was formed by injecting 10 mL of sterilized 1.5% CMC in saline. An infection was induced by inoculating 2×10^6 colony-forming units of MRSA W3275 per pouch along with CMC. The local infection model formed after 4 days of incubation. In addition, six rats were injected with only 10 mL of saline without CMC and bacterial inoculum (sham model) as negative control groups. Twenty-four rats with the local infection were randomly divided into four groups (three therapy groups and one fibroblast control group), with six rats per-group. Each of the three therapy groups was given a different number of