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Metallic nanoparticles to eradicate bacterial bone infection

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Abstract

Treatment of osteomyelitis by conventional antibiotics has proven to be challenging due to limited accessibility to this unique location. Inorganic routes against bacterial infection have been reported for external and topical applications, however in vivo application of these antimicrobials has not been fully explored. Targeted delivery of metallic nanoparticles with inherent antimicrobial activity represents an alternative means of overcoming the challenges posed by multidrug-resistant bacteria and may potentially reduce overall morbidity. In this study we utilized silver–copper–boron composite nanoparticles in an attempt to eradicate *S. aureus* bone infection in mice. Our results demonstrate effective response when nanoparticles were administered via i.v. or i.m. route (1 mg/kg dose) where 99% of bacteria were eliminated in an induced osteomyelitis mouse model. The 1 mg/kg dose was neither toxic nor produced any adverse immune response, hence it is believed that metallic nanoparticles present an alternative to antibiotics for the treatment of bone infection.

Key words: Osteomyelitis; In vivo; Nanoparticles; Ag-cu-B; Animal model

Osteomyelitis management involves long-term antibiotic therapy. Surgical intervention is highly recommended for chronic osteomyelitis.[1] Difficulties in treating osteomyelitis are believed to stem from the sheltered physiological environment offered to the bacteria and poor accessibility to the immune system and to therapeutic agents.[2,3] Several suggestions to control the amount of antibiotics by either novel delivery systems such as chewable tablets or localized delivery of the antimicrobials have been described.[4–7]

Metal or polymeric implants with or without drugs have been studied to identify alternatives for osteomyelitis treatment.[8–10] Therapeutic modalities on animal models (e.g. sheep,[11] goat,[12] pig,[13] dog[14,15] and mouse[16–23]) to manage chronic osteomyelitis have been developed, however many more studies are still needed to identify a modality with low recurrence rate and drug resistance. The recurrence rate of osteomyelitis is more than 50% following three months of antibiotic treatment.[24]

The oligodynamic activity of metals provides a valuable alternative to the use of systemic antibiotics. Although silver seems to be the favorite antimicrobial metal,[25–28] there are several shortcomings associated with its use as a sole agent. Silver has a short-term antimicrobial activity that requires its continuous re-application and also the fact that silver requires an aqueous environment that produces the active ionic form to display its effect.[1,29] Most studies have examined the antimicrobial activity of Ag ions or nanoparticles in vitro, few have reported the toxicity of Ag using in vivo models.[30–34] Studies have shown that Ag-Cu is more effective as an antimicrobial agent compared to Ag lone or Cu alone.[35] The antimicrobial effectiveness of Ag-Cu complex is also however, limited by the

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rapid Cu oxidation.[35] We have found (data not reported here) that the Ag ion release is maintained for a longer period when boron is introduced to the Ag-Cu complex. The longevity of antimicrobial effectiveness for Ag-Cu-B is greater than that of Ag-Cu. This could be attributed to the B anticorrosive properties that delay the Cu oxidation.[36] The objective of our study is to report a silver–copper–boron (Ag-Cu-B) composite nanoparticles as an alternative for osteomyelitis management using in vivo model system. The Ag-Cu-B complexing overcomes the shortcomings associated with silver and silver copper.

Antimicrobial function of Ag-Cu-B is reported for the first time in in vivo as therapeutic agent for bone infection. An osteotomy mouse model was developed. The development of infection was assessed by microbiological technique. The efficacy of the local application of Ag-Cu-B nanoparticles against an antibiotic was assessed.

Methods

Synthesis and characterization of Ag-Cu-B nanoparticles

Particles were synthesized as reported in Ref.[37] Briefly, 0.1 Molar salt of copper (II) sulfate, silver nitrate and boric acid were prepared in 100 ml deionized water in a ratio of 70:20:10 (Ag:Cu:B). Salt solution was heated to 90 °C in a triple neck flask with constant mixing by using Teflon rod fixed with homogenizer under the fume hood. Flask was purged with argon gas. Continuous stream of argon was used throughout the reaction. 8 M NaOH was added drop wise from the side neck of flask until the formation of black precipitate. The solution was heated for 20 min until the precipitate turned to grayish black. The precipitate formed was washed repeatedly three times or more in deionized water and centrifuged at 4000 rpm for 10 min for each wash. Furthermore 100 mM of lactic acid treatment for 10 min was used to break the nanoparticles in smaller size, particles were collected by centrifugation and washed 3 times or more with water to remove the lactic acid from samples. The final pellet was sonicated (Branson Sonifier–450) for 1 h on ice to prevent the rise in temperature associated with sonication. After sonication the sample was filtered through Whatman filter paper, freeze-dried and stored in 20 ml airtight glass vial with a screw cap. Prior to using the nanoparticles, frozen particles were weighed and suspended in deionized water, sonicated and used immediately.

Ag-Cu-B nanoparticles were characterized by the XRD-technique (Agilent Technologies Oxford Gemini X-Ray Diffractometer). The XRD technique (Molybdenum source: Voltage 50 kV and with 30 mA current and λ Mo = 0.709 °A) was used to study the phase formation in and morphology of the Ag-Cu-B nanoparticle. Transmission electron microscopy was done with FEI Talos F200C, images were obtained at 200 KV. Atomic force microscopy (AFM) Bruker Model was employed to confirm the height of nanoparticles. Scanning was done at 1 Hz. The hydrodynamic size of nanoparticles was measured with a Nano-sizer NZS (Malvern). Scanning Electron Microscopy SEM JEOL6400 -Oxford EDS unit analyzed the surface morphology and elemental analysis.

S. aureus XEN-36 strain

S. aureus XEN-36 strain which was derived from the parental strain *S. aureus* ATCC 49525 was obtained from Caliper Life Sciences, USA which possesses a stable copy of the modified photorhabdus luminescence lux ABCDE operon at a single integration site on a native plasmid, stock was stored in glycerol at −80 °C. This kanamycin resistant *XEN-36* was streaked on tryptic soy agar plate with the recommended dose of kanamycin (200 μg/ml) as per guidelines of Caliperlife Sciences. Preparation of bacterial cultures was carried out essentially as previously described.[38] Bacterial colonies grown on T-soy agar plate were inoculated into 5 ml of T-soy broth and cultured stationary overnight with aeration. The overnight grown bacterial culture was then sub-cultured at 1:5 ratio and grown to mid-log phase for another 2 h with shaking at 200 rpm and was stopped when O.D. reached to 0.5 at 600 nm wave length. Colony forming units (CFU's) count was estimated at 0.5 O.D. by harvesting bacterial cells with centrifugation at 4000 rpm for 20 min and the pellet was re-suspended in 5 ml of PBS pH 7.4. Serial dilutions were performed in sterile PBS and 100 μl aliquots were plated on T-soy agar plates containing 200 μg/ml kanamycin. The number of bacterial colony forming units (CFUs) were enumerated after overnight incubation at 37 °C.

XEN-36- S. aureus growth on braided silk suture

A bacterial suspension of *XEN-36* containing 200 μg/ml of kanamycin was adjusted with Tryptic-Soy to O.D. 0.5 at 600 nm. A braided silk suture 5–0 size (SMI, REF.NO. 8151516, LOT NO. 110623) was tumbled in this bacterial suspension for 45 or 150 min, it was gently removed and air dried for 5 min by keeping on sterile Whatman filter paper and cut to a length of 2 cm. Further it was chopped into small pieces in 1 ml of PBS and followed by homogenization by tissue homogenizer. Serial dilutions of silk suture homogenate were performed in sterile PBS and 100 μl was plated on T-soy agar plates with kanamycin. Number of CFUs were enumerated after overnight growth at 37 °C in incubator.

Mouse strain

Female BALB/c mice were purchased from Harlan Olac (Biocester-UK). Mice were bred in the animal care facilities of the College of Medicine and Health Sciences United Arab Emirates University, and maintained in filter-topped isolator cages. Mice were housed under controlled dark and light cycle of 12 h each in groups of 5–6 mice per ventilated cages and post-surgery or treatment they were rehoused in same groups. They were fed standard diet with food and water ad libitum. All studies involving animals were conducted in accordance with and after approval of the animal research ethics committee of the College of Medicine and Health Sciences, United Arab Emirates University.

Osteotomy

8–12 week-old BALB/c female mice were anesthetized with a combination of xylazine (10 mg/kg of body wt.) and ketamine (100 mg/kg of body wt.). Xylazine and ketamine ratio were made