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Screening of invitro activity of antimicrobial and antibiofilm property of Mesenchymal stems cells against MDR gram negative organism isolated from urinary tract infections in tertiary care hospital

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Abstract

Introduction & Objective: Recent studies had showed that Mesenchymal Stem Cells (MSCs) have beneficial effects on bacterial infections. Treatment with MSCs has proven bacterial clearance. This study was undertaken to study the in vitro activity of antimicrobial and antibiofilm activity of stems cells against gram negative multidrug resistant organism from urinary tract infections. Today, because systemic infections such as urinary tract infection (UTI) affect even pediatric patients, antibiotic resistant bacteria have become a constant clinical challenge. In the present study, a total of 1054 urine samples were collected from pediatric patients over 18 months. From these samples, 510 isolates of pathogenic bacteria were collected using HiCrome UTI agar. Antibiotic sensitivity tests of isolates were performed using the Kirby-Bauer method. Two Gram-positive bacteria (Enterococcus faecalis and Staphylococcus aureus) and 7 Gram-negative bacteria (Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca, K. pneumoniae, Proteus vulgaris and Pseudomonas aeruginosa) were isolated. Antibiograms of isolated bacteria were ascertained using antibiotics of 4 classes: aminoglycosides, β-lactams, fluoroquinolones and 2 stand-alones (co-trimoxazole and nitrofurantoin). Based on percent values of antibiotic resistance, isolated bacteria were (in decreasing order of number of isolated isolates): E. coli (109)>S. aureus (65)>E. faecalis (82)>E. aerogenes (64)>C. freundii (41)>P. aeruginosa (32)>K. pneumoniae (45)>K. oxytoca (50)>P. vulgaris (22). Surveillance results show that MDR isolates of 9 pathogenic bacteria were prevalent in the environment around the hospital. Thus, revisions to the antimicrobial stewardship program in this area of the country are required to increase clinician confidence in empiric therapy, which is often used for UTI cases. Acute lung injury (ALI) is a major cause of acute respiratory failure in critically ill patients. Bacterial pneumonia is the most common cause of ALI [17]. Recent studies have demonstrated that BM-derived MSCs reduce lung injury in experimental models of lipopolysaccharide (LPS)-induced ALI in mouse [18, 19] and in an ex vivo-perfused human lung [20]. In addition, other in vitro and in vivo studies have provided evidence for the beneficial effects of MSCs in the treatment of LPS- or bacteria-induced sepsis. In two mouse models of sepsis following cecal ligation and puncture (CLP), i.v. MSCs reduced total bacterial counts in the blood and peritoneal fluid [21, 22]. These survival benefits were explained in part by the immunomodulatory properties of MSCs, but the actual mechanism of enhanced bacterial clearance was not clearly identified. Although a recent publication by Mei et al. [23] showed that the improvement in bacterial clearance in MSC-treated septic mice following CLP could be in part explained by enhanced phagocytic activity of host immune cells, it is not known yet whether human BM-derived MSCs possess direct antimicrobial activity.

Thus, the primary hypothesis for these studies was that human MSCs might express direct antimicrobial activity through the secretion of antimicrobial peptides. We examined the effect of human MSCs on bacterial growth in vitro. Expression of different antimicrobial peptides was investigated using reverse transcription polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry. Following stimulation with live *Escherichia. coli*, human MSCs produced one candidate antimicrobial peptide, LL-37, which was subsequently found to be responsible for antimicrobial activity in vitro. To determine if the secretion of LL-37 by MSCs would alter bacterial clearance in vivo, we tested BM-derived human MSCs in an *E. coli* pneumonia model in mice. Treatment with human MSCs, given 4 hours later, resulted in a significant reduction of *E. coli* colony-forming unit (CFU) in the lung homogenates (LHs) and the bronchoalveolar lavage (BAL) fluids. The effect was blocked with a neutralizing antibody to LL-37 demonstrating that human MSCs possessed antimicrobial activity, which is explained in part by the secretion of LL-37.

Method: The samples will be processed according to standard protocol following standard guidelines. All the isolates obtained will be identified by standard guidelines. Total of 50 isolates were collected. The antibiotic susceptibility testing will be done for all the isolates by Kirby Bauer disc diffusion method following CLSI guidelines. All the isolates are screened for production of biofilm by tissue culture plate method. The antimicrobial activity of mesenchymal stem cells was done by micro broth dilution method.

Results: Among the 50 gram negative isolates 22 (44%) were Pseudomonas species 12 (24%) were E coli 8 (16%) were Klebsiella spp and 8 (16%) were Proteus species. Among the 50 isolates 32 (64%) were multi drug resistant to the antibiotics tested. Among the 50 isolates 43 (86%) produced biofilm of which 28 (65%) were strong producer 8 (18%) were moderate biofilm producer and 7(16.27%) were weak biofilm producers. All 43 isolates showed sensitivity for the mesenchymal

stem cells with MIC range of 32-0.25?g.

Conclusion: So far only very few or no studies have been reported on anti-biofilm activity of mesenchymal stems cells. From our study stem cell, therapy with MSC will be effective and alternate for antibiotic resistance in chronic urinary infection there by can serve as therapeutic options for treating drug resistant organisms.

Biography

C Anitha has completed her PhD from Dr ALM PGIBMS, University of Madras, India. She is currently working as Assistant Professor of Microbiology in Meenakshi Medical College Hospital and Research Institute. Her areas of interest are Biofilm studies using Confocal Laser Scanning Microscope, Antimicrobial resistance and rare Infectious diseases. She has published more than 30 papers in reputed journals and also has 15 Data sequences submitted in PUBMED/NCBI Genebank. She is also serving as an Editor, Associate Editor, Editorial Board Member and Review Board Member of reputed journals.

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