Clinical Significance of Joint Fluid Lactic Acid Levels: What Does It Mean in Septic Arthritis?

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INTRODUCTION:

Septic arthritis is an important healthcare system concern that warrants urgent treatment to eradicate bacteria and reduce intra-articular inflammation destructive to cartilage. Clinicians routinely include lactic acid levels to rule in or rule out septic arthritis. However, no real clinical or scientific significance of lactic acid level in the context of bacterial septic arthritis has been taught or explained. The purpose of this rigorous investigation is to define the clinical significance of lactic acid and its upstream metabolic pathway, glycolysis. Once the proposed study is completed, accurate interpretation of lactic acid levels would be possible.

METHODS:

Overall approach: We conducted measurement of glycolytic pathway metabolite lactate and its enzyme, GAPDH, in MRSA and MRSA-infected macrophages. We also established a murine septic arthritis model that showed massive synovial inflammation and loss articular cartilage after MRSA knee joint infection. Lastly, we used an inhibitor of glycolytic pathway that is linked to lactate production in MRSA, macrophages, and joint cells.

In vivo MRSA knee infection: All animal experiments were approved by the Institutional Animal Care and Use Committee. MRSA (4×10^6 CFU/10 µl) were intraarticularly injected under the patella. Control animals were injected with the same volume of DPBS. For the systemic or intra-articular dimethyl fumarate (DMF) treatment groups, vancomycin (30 mg/kg) and rifampin (20 mg/kg) were subcutaneously injected using a U-100 Micro-Fine Insulin Syringe for 3 days following infection of the knee joint with MRSA (8×10^6 CFU/10 µl). The next day, DMF was subcutaneously (14 mg/kg for the systemic treatment group) or intraarticularly (70 µg/joint for the intra-articular treatment group) administered.

Knee joint tissue sections were then embedded in paraffin and stained with hematoxylin and eosin (H&E) and safranin O (SAF O) according to the manufacturer's instructions. H&E-stained images were used for inflammation scoring and to characterize synovial hyperplasia and synovial cellularity according to previous guideline. SAF O-stained images were used to generate OARSI scores and to characterize osteophyte formation, proteoglycan depletion, bone erosion, and bone formation according to previous guidelines.

MRSA Growth Assay: Different concentrations of dimethyl fumarate (DMF; 140, 420, and 700 µg/ml) and vancomycin (2.5 mg/ml) were added to LB (200 µl; Invitrogen) medium containing oxacillin (6 µg/ml; Sigma-Aldrich Co.) in 96 well plates (BD Biosciences); DMSO was used as the negative control. MRSA (4×10^6 CFU) was seeded into the medium and absorbance (600 nm) was measured using one manufacturer's cell imaging multimode reader in a 35°C incubator at intervals of 1 hour for a total of 24 hours. Data were analyzed.

Statistical analysis: All experimental data was analyzed using one- and two-way analysis of variance (ANOVA).

RESULTS:

We confirmed that the production of inflammatory markers and glycolysis products increases in MRSA-infected bone and immune cells using RNA-sequencing analysis. We observed the accumulation of inflammation and lactate intra-articularly in a clinically relevant murine model of MRSA-induced septic arthritis and verified these findings in MRSA-infected human synovial and cartilage tissues. We verified that high concentrations of lactate and pro-inflammatory markers persisted within the joint space even after conventional antibiotic treatment. Adjuvant therapy with glucose uptake inhibitors in combination with antibiotics was ineffective, but Di-methyl-fumrate (DMF), which selectively targeting the glycolysis pathway, was more effective in reducing the production and secretion of pro-inflammatory markers than other tested drug candidates. Moreover, DMF inhibited the MRSA proliferation and biofilm formation via bacteriostasis and improved the efficacy of vancomycin, which is ineffective against intracellular MRSA, by successfully hindering the proliferation of intracellular MRSA. Both systemic and intra-articular adjunctive DMF administration after antibiotic treatment yielded more favorable prognoses inclusive of cartilage and bone protection, inflammation reduction, and reduced lameness in the setting of murine septic arthritis.

DISCUSSION AND CONCLUSION:

In summary, lactic acid is produced by bacteria and joint inflammatory cells in septic arthritis. The elevation of lactic acid cannot distinguish its bacterial or joint cells. It is co-incidental that bacteria and joint cells share the same glycolysis pathway for bacterial proliferation and host cell inflammation. We were able to propose a new therapeutic opportunity. Glycolysis targeting with a fumarate analog, di-methyl-fumarate (DMF) not only inhibited MRSA bacterial proliferation but also protected articular cartilage from glycolysis-mediated joint inflammation. In conclusion, lactate is a biomarker for knee inflammation and its metabolic pathway is an excellent therapeutic target in the context of septic arthritis.

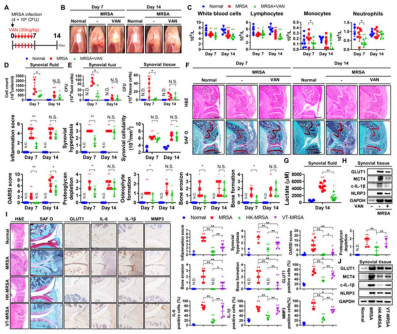

Figure 1. MRSA-induced septic arthritis incites intra-articular inflammation and lactate production that persists after vancomycin treatment

A. C57BL/6J mice were subcutaneously treated with vancomycin (30 mg/kg) for 6 days following MRSA (4×10^6 CFU) infection and sacrificed at 7 and 14 days (n = 4-8 per group).

B. Physiological changes were identified and representative images were generated.

C. Blood was collected and complete blood counts (CBC) were measured.

D and E. Infiltrating immune cell counts within synovial fluid and MRSA synovial fluid bioburden were quantified. MRSA bioburden in synovial tissue was quantified.

F. Paraffin-embedded tissues were sectioned and measured at 7 and 14 days with respect to inflammation score, synovial hyperplasia, synovial cellularity, OARSI score, proteoglycan depletion, osteophyte formation, bone erosion, and bone formation (Scale bar: 1,000 μm).

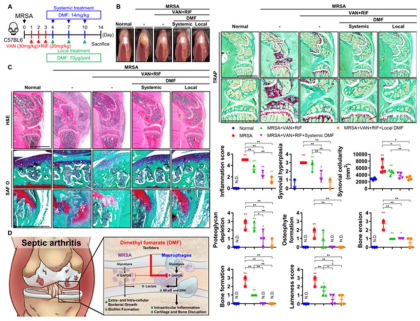

Figure 2. Adjuvant DMF treatment with antibiotic treatment improves the prognosis o MRSA-induced septic arthritis

A. C57BL/6J mice were subcutaneously treated with vancomycin (30 mg/kg) and rifampin (2 mg/kg) for 3 days following MRSA (8×10^6 CFU) infection, followed by systemic (14 mg/kg) o local (70 μg/joint) DMF treatment at 3 days interval for a total of 3 times (n = 4 per group).

B. Physiological changes were identified.

C. Paraffin-embedded tissues were sectioned and measured with respect to inflammation score synovial hyperplasia, synovial cellularity, proteoglycan depletion, osteophyte formation, bon erosion, and bone formation (Scale bar: 2,000 or 500 μm). Bone resorption by osteoclasts wa measured by tartrate-resistant acid phosphatase (TRAP) staining (Scale bar: 2,000 μm). Lamenes score was also measured.