Oxalate impacts macrophage metabolism and immune response to uropathogenic *E. coli* infection

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Introduction & Objective

INTRODUCTION

- Urinary stones are suggested to be associated with oxalate and bacterial infection.
- *Escherichia coli* (*E.coli*) has been isolated from human urine and urinary stones composed of oxalate.[1-2] It has also been shown to promote calcium oxalate (CaOx) crystallization.[3]
- Stone formers have a high incidence of urinary tract infection (UTI) and increased inflammation.[4-5]
- Oxalate and crystals have been shown to induce inflammation (i.e. Monocyte Chemoattractant Protein-1 chemokine release) in renal epithelial cells[6-7].
- The mechanisms leading to UTI in kidney stone patients are not well defined.
- We have previously determined that 1) monocyte cellular energetics is reduced in CaOx kidney stone formers, and 2) oxalate alters redox homeostasis and cellular energetics in monocytes (**Figure 1**).

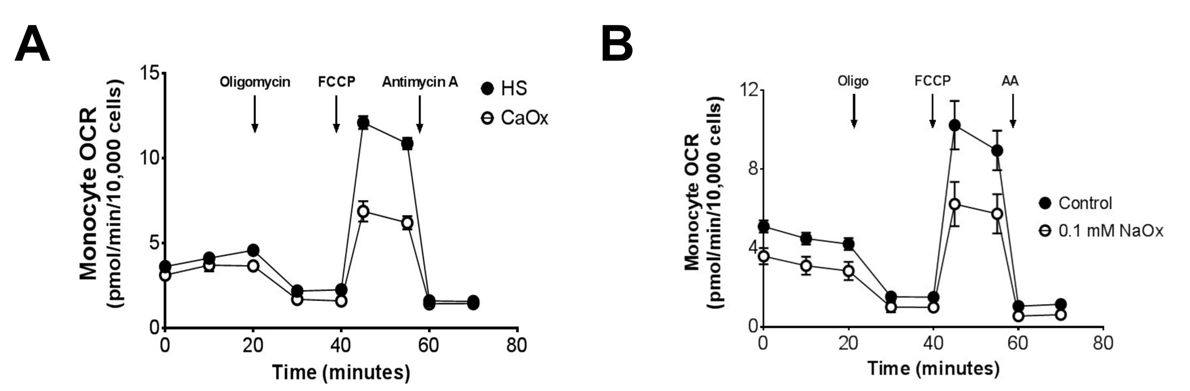
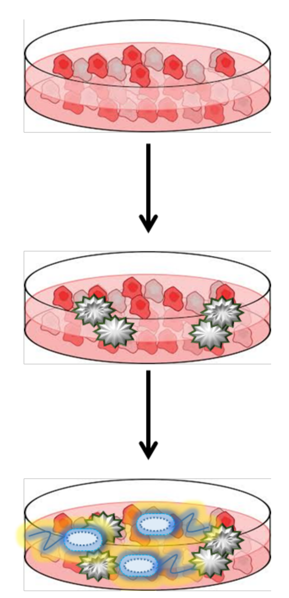


Figure 1: Cellular energetic profiles in (A) monocytes isolated from healthy subjects (HS; black circle) and CaOx SF (white circle) and (B) monocytes from HS (black circle) treated with sodium oxalate. Results are presented as mean ± se; n=5-6 replicates per group.

OBJECTIVE

—To determine the impact of oxalate on macrophage metabolism, redox status, and anti-bacterial response to uropathogenic *Escherichia coli* (*E. coli*) infection.

Methods



THP-1 Cells (Monocytes)

- Treated with sodium oxalate (NaOx) (50 μM, 48 hours)
- Differentiated into macrophages (PMA, 200 nM, 48 hours)

THP-1m (Macrophages)

- ± CaOx Crystals (50 μM, 24 hours)
- ± Uropathogenic *E. coli* strain (CFT073; 1:2, 1-8 hours)

Methods

- Cell Viability & Proliferation: CCK8 and LDH assays were performed to assess macrophage proliferation and viability, respectively.
- Mitochondrial Membrane Potential: was measured using TMRE, a fluorescence dye.
- Oxidative Stress: Mitochondrial superoxide was measured using a fluorescence mitochondrial superoxide dye and total ROS was assessed using DCF staining.
- Cellular Energetics: A Seahorse XF Analyzer was used to assess metabolism.
- Infection assay: The colony forming unit (CFU) count was determined by counting the amount of bacteria present in cell lysates following treatment.

Results

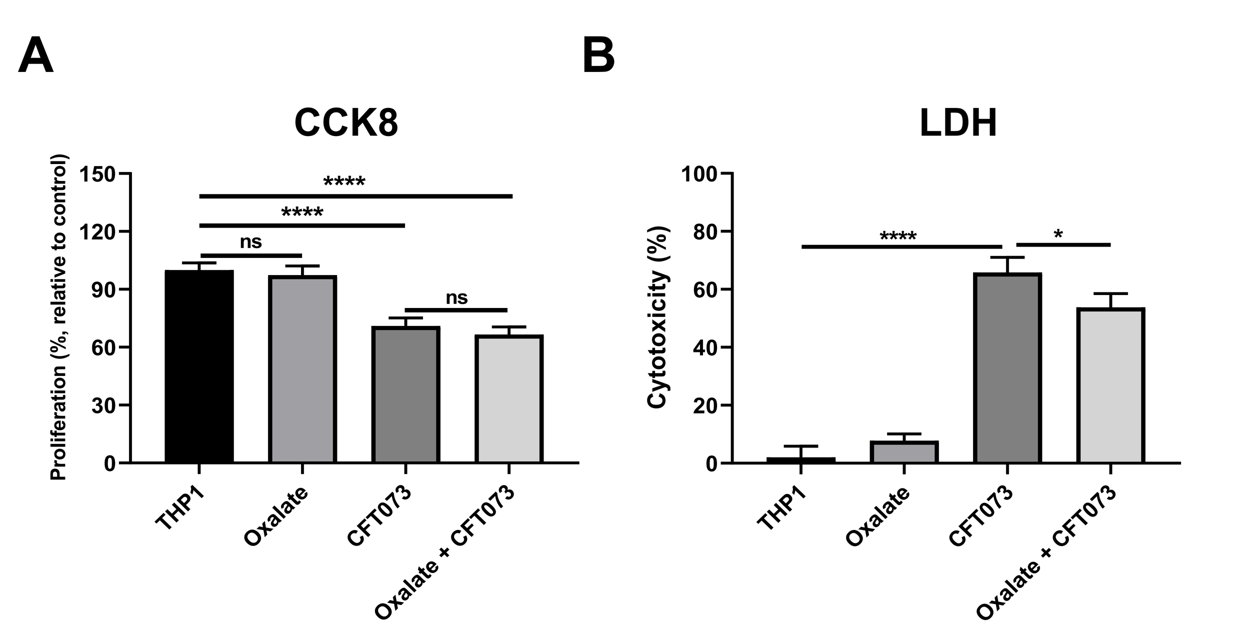


Figure 2: The effect of oxalate and CFT037 on THP-1 macrophage (A) proliferation and (B) viability. n=5 per treatment group. *p<0.05,****p<0.0001.

Results

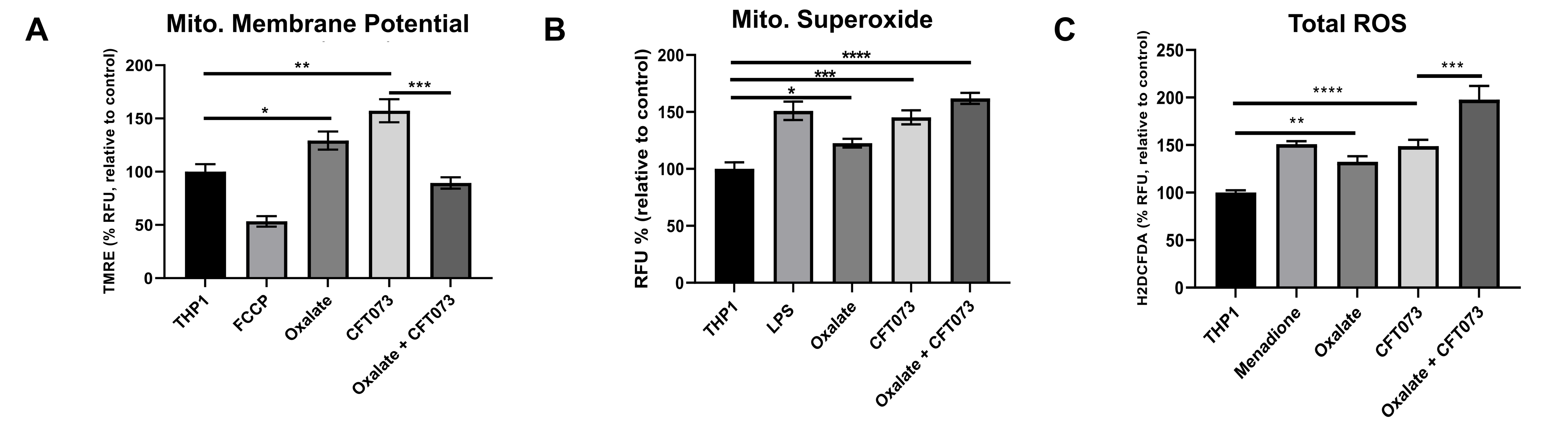


Figure 3: The effect of oxalate and CFT037 on THP-1 macrophage (A) mitochondrial membrane potential, (B) mitochondrial superoxide, and (C) total reactive oxygen species (ROS) levels. Positive controls: FCCP, LPS, and Menadione. n=5 per treatment group. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

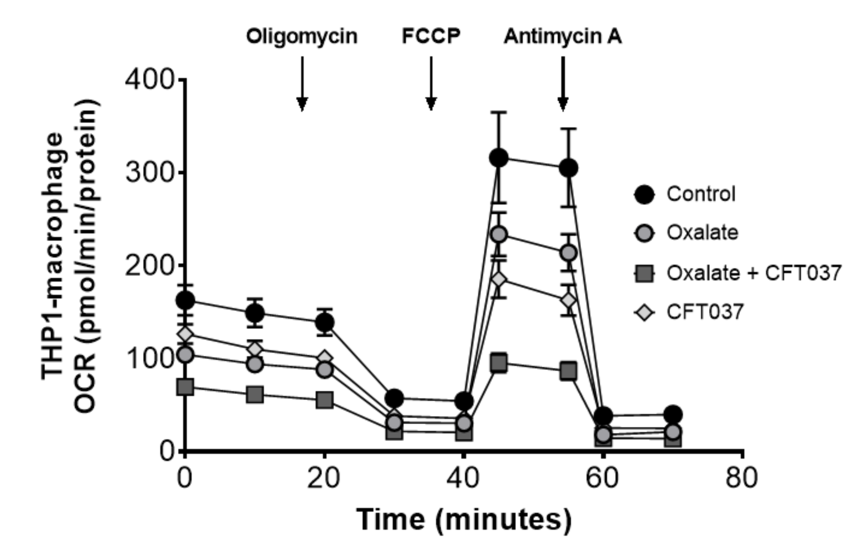


Figure 4: The effect of oxalate and CFT037 on THP-1 macrophage cellular energetics. Results are presented as mean ± se; n=5-6 replicates per group.

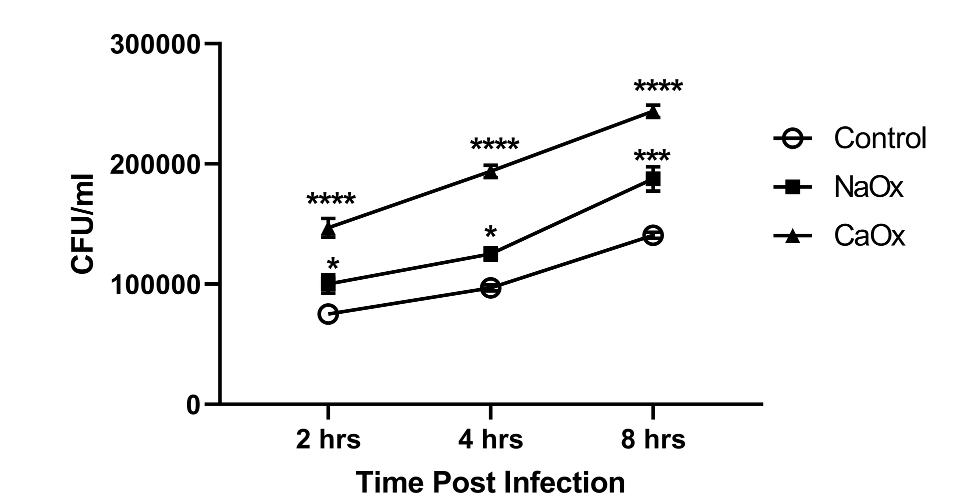
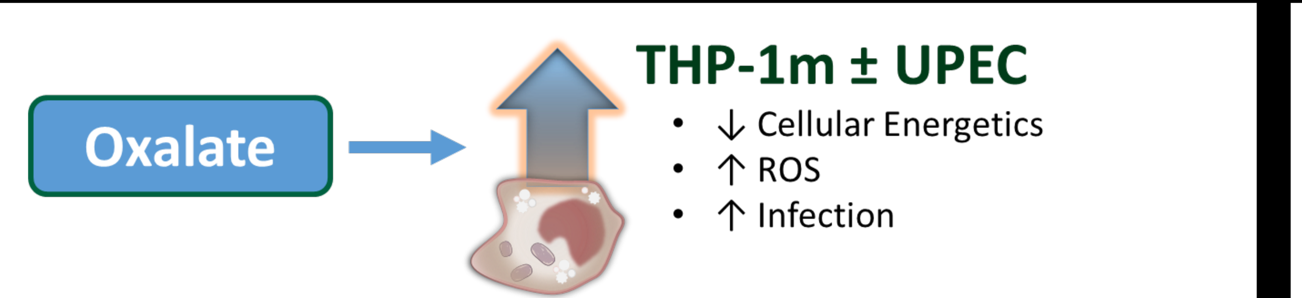


Figure 5: The effect of oxalate on the anti-bacterial response of THP-1 macrophages over time. *p<0.05, ***p<0.001, ****p<0.0001.

Discussion



- These findings show oxalate impacts macrophage redox status, metabolism, and anti-bacterial response.
- Elucidating the role of CaOx crystals on macrophages during KS disease is critical and should provide new insight to the field since stone formers have increased KS recurrence and associated UTIs.

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Support & Contact

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