NLRP3 inflammasome inhibitor attenuates cisplatin-induced macrophage foam cell death in an *in vitro* model of atherosclerosis

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BACKGROUND

Cisplatin (CPDD) is a commonly prescribed chemotherapeutic agent that is FDA approved to treat testicular, bladder, and ovarian cancers. While CPDD can be effective, patients who have undergone CPDD treatment are at higher risk for long-term cardiovascular morbidity and mortality, specifically acute coronary syndrome (ACS).[1] The mechanisms underlying this increased risk are largely unknown. However, recent studies have suggested that CPDD's long-term toxicity may be mediated by the pro-inflammatory activation and death of macrophage foam cells (MFC), which causes atherosclerotic plaque destabilization and subsequent coronary artery thrombosis.[2] Previous research has implicated the NLRP3 inflammasome—a protein complex critical for innate immunity—as well as the downstream cytokine IL1-β in atherosclerosis development.[3,4] Thus, **we hypothesized that the NLRP3 inflammasome is involved in cisplatin-induced plaque rupture.** The novel drug OLT1177 (dapansutrile) is known to inhibit the NLRP3 inflammasome and has previously been shown to be cardioprotective.[5]

METHODS

Human monocyte THP-1 cells were differentiated into macrophages with 150 nM of phorbol 12-myristate 13 acetate (PMA) for 48 hours, then further differentiated into macrophage foam cells (MFCs) with 100 µg/mL of Oxidized LDL in serum-free media for 24 hours. Differentiation was assessed via Oil Red O (ORO) staining. A dose-response curve was obtained for MFCs treated with cisplatin.

MFCs plated in a 96-well plate were treated with 50µM or 100µM of OLT1177 alone, 25µM of cisplatin alone, or both. Following 24 hours of incubation, the methyl thiazolyl diphenyl-tetrazolium bromide (MTT) assay was used to assess the cell viability of MFCs subjected to cisplatin and OLT1177 (Figure 1). The MTT assay quantitatively assesses cellular metabolic activity to measure cell viability.

RESULTS

THP-1 cells treated with PMA and oxidized LDL demonstrated a 25% increase in lipid uptake compared to THP-1 cells treated with PMA alone (Figure 1). Treatment of MFCs with cisplatin exhibited a dose-response relationship (Figure 2). Resulting in roughly 20% cell death, 25µM cisplatin was used for subsequent experiments to mimic a clinically relevant dose of cisplatin. Cisplatin-treated MFCs pre-treated with 50µM and 100µM of OLT1177 showed a decrease in cell death from 16% to 9% and 8.5%, respectively (Figure 3).

CONCLUSION

Treatment of MFCs with the NLRP3 inflammasome inhibitor OLT1177 attenuated cisplatin-induced foam cell death. While these results did not reach the threshold for statistical significance, the data reveal a trend that suggests that OLT1177 may protect against cisplatin-induced atherosclerotic plaque destabilization. Additional work is needed to determine statistical significance as well as the clinical relevance of this trend.

NLRP3 inflammasome inhibitor OLT1177 may protect against cisplatin-induced macrophage foam cell death

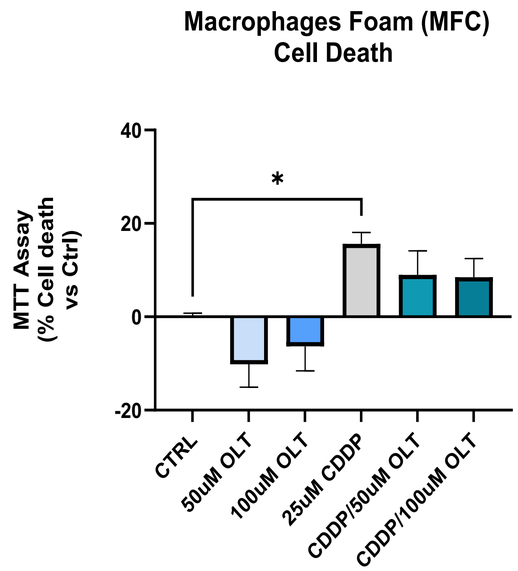

FIGURE 3. OLT1177 decreases CDDP-induced MFC death. Cell death was measured by the MTT assay. Results expressed as % cell death compared to untreated cells (mean ± SEM). *P<0.05

DISCUSSION

The results from the CANTOS study indicate that IL-1β—a cytokine downstream of NLRP3 activation—is involved in atherosclerosis.[3] Our preliminary results indicate that NLRP3 inhibition by the experimental drug OLT1177 attenuates pro-inflammatory macrophage foam cell death, which may be involved in cisplatin-induced atherosclerotic plaque destabilization. Given its established safety profile[5], OLT1177 represents a promising treatment to reduce the cardiovascular burden of cisplatin treatment.

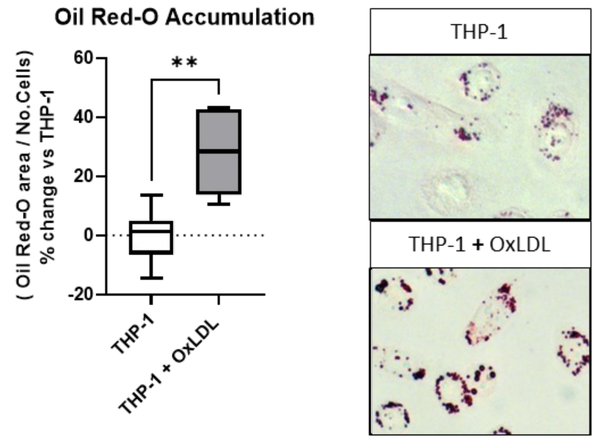

FIGURE 1. Foam cell formation assessed with Oil Red-O Staining. Oil Red-O is a dye that stains intracellular lipids. Increased lipid uptake is characteristic of foam cell formation. Results expressed as mean +/- SEM of Oil Red-O accumulation in THP-1 cells treated with PMA and Oxidized LDL. **P<0.01

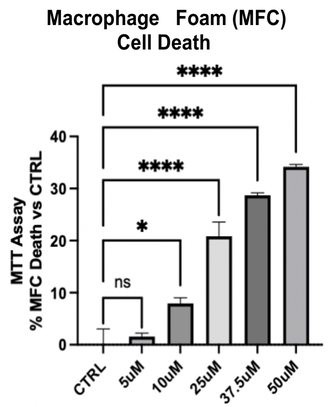

FIGURE 2. Dose-Response Curve of Cisplatin Treatment on MFCs. MFCs were treated with a range of cisplatin concentrations. Cell death was measured by the MTT assay. Results expressed as % cell death compared to untreated cells (mean ± SEM). *P<0.05, ****P<0.0001

Disclosures

- **Dr. Salloum serves on the Advisory Board for NovoMedix, LLC. All other authors declared no conflict of interest**
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Citations

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