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Reactive Aldehyde Species (RASP) Inhibitors Sequester MAA-Adducts and Reduce Pro-Inflammatory Cytokines

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REACTIVE ALDEHYDE SPECIES (RASP) INHIBITORS SEQUESTER MAA-ADDUCTS AND REDUCE PRO-INFLAMMATORY CYTOKINES

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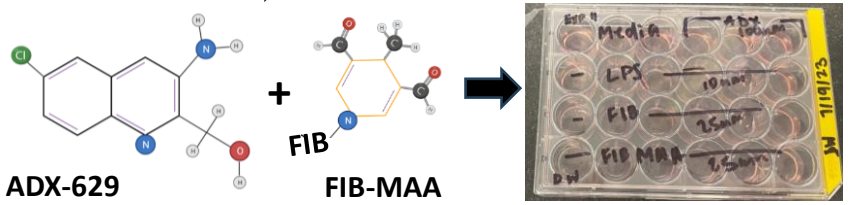
Background and Purpose

- Post-translational modifications of self-proteins have been implicated in the pathogenesis of many diseases, notably Rheumatoid Arthritis (RA), alcoholic liver disease, and ulcerative colitis. Two biomolecules - malondialdehyde (MDA) and acetaldehyde (AA) - are commonly seen in inflammatory states and are signs of oxidative stress and lipid degradation and are shown to be elevated in RA. MDA and AA have been shown to interact and form a modified protein labeled malondialdehyde-acetaldehyde adduct or MAA. This protein adduct contributes to inflammation through inducing immune cells to generate pro-inflammatory cytokines such as interleukin-1 (IL-1), tumor-necrosis factor-α (TNF-α), and IL-6 . Consequently, MAA increases T-cell specific responses and circulating autoantibodies.
- MDA and AA are reactive aldehyde species (RASP) that function by covalently bonding to molecules and disrupting their cellular processes. Recently RASP inhibitors (ADX-629 and ADX-246) became available that have been shown to prevent the formation of MAA adducts by covalently binding and sequestering MDA and AA in a mouse model of alcoholic liver disease. Through inhibition of MDA and AA these inhibitors prevented the release of key inflammatory cytokines **and** protected animals from progressive liver damage. While these experiments demonstrated the binding of RASP inhibitors to MDA and AA prior to MAA formation, they did not determine the capacity of these agents to scavenge pre-formed MAA protein adducts.
- Therefore, the purpose of this study was to determine if RASP inhibitors sequester MAA-adducts and block the subsequent cellular release of pro-inflammatory cytokines.

Methods

- Human monocytic cells (U-937 cell line) were pipetted into a 24 well plate at a concentration of 1 x 10^6cells/mL. Cells were activated to professional macrophages using phorbol 12-myristate 13-acetate (PMA) and media was replaced every 24 hours for two days.
- After 48 hours, U-937 cells were pre-treated with decreasing doses of ADX-629 or ADX-246 (200µM, 100µM, 50µM, 10µM, 1µM, 0µM) for 30 minutes.
- Macrophages were then incubated with 25µg/mL of fibrinogen (FIB) or MAA-modified fibrinogen (FIB-MAA) for 24 hours at which time supernatants were collected for analysis.
- Supernatants were evaluated for levels IL-6 and MCP-1 using commercially available Biolegend ELISA MAX kits.
- Additionally, cells were collected and tested for membrane integrity using a lactate dehydrogenase (LDH) assay.



Results

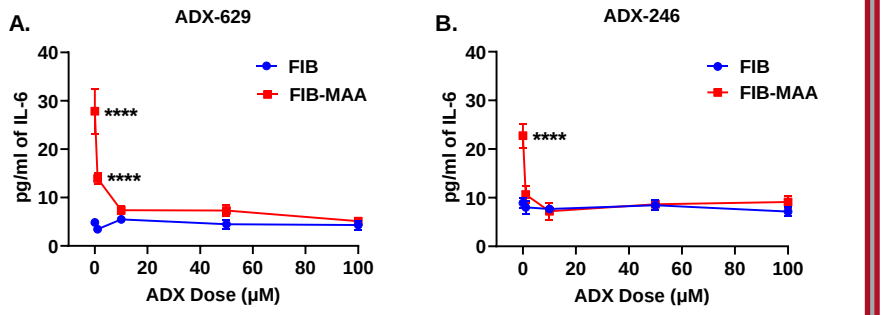


Figure 1. ADX Prevents the Release of IL-6 following Fibrinogen-MAA Stimulation. In the absence of RASP inhibition, FIB-MAA stimulation of cells resulted in a significantly higher IL-6 release ($p<0.001$) compared to FIB alone. IL-6 release was significantly reduced with only 1 µM of ADX-629 and fell to background levels with drug concentrations exceeding10 µM (**Figure 1A**). ADX-246 demonstrated similar results only much more rapidly (**Figure 1B**). (N=6)

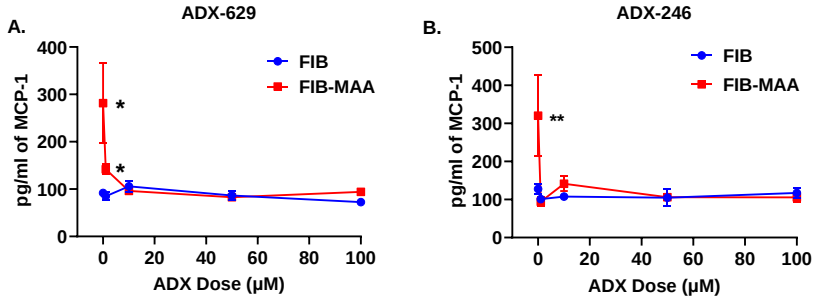


Figure 2. ADX Prevents the Release of MCP-1 following Fibrinogen-MAA Stimulation. In the absence of RASP inhibition, FIB-MAA stimulation of cells resulted in a significantly higher MCP-1 release ($p<0.05$) compared to FIB alone. MCP-1 release was significantly reduced with only 1 µM of ADX-629 and fell to background levels with drug concentrations exceeding10 µM (**Figure 2A**). ADX-246 demonstrated similar results only much more rapidly (**Figure 2B**). (N=6)

Results

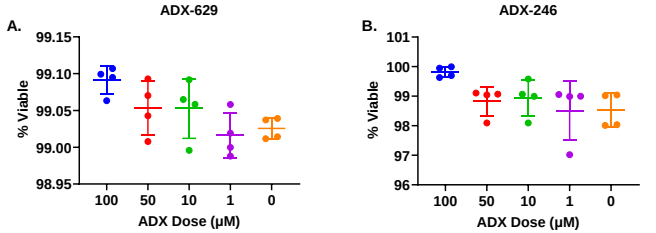


Figure 3. ADX is not Toxic to Macrophage. Following stimulation of macrophage with FIB-MAA in the presence of doses of ADX-629 (**Figure 3A**) or ADX-246 (**Figure 3B**) there were no toxic effects demonstrated as determined by the Lactate dehydrogenase assay (LDH). N=6

Discussion

- MAA modified self proteins can render harmful effects in patients with RA, the ability to inhibit their deleterious effects could have notable clinical implications.
- This research confirms that RASP inhibitors have the ability to bind and sequester pre-formed MAA in addition to binding AA and MDA.
- Results show that ADX-246 is much more efficient at blunting the MAA response.
- The ability of ADX RASP inhibitors to bind pre-formed MAA, could lead to its potential use as a disease modifying anti-rhematic drug in numerous inflammatory diseases, particularly in autoimmune diseases as inflammation is a hallmark of so many diseases.
- Additionally, MAA is implicated in other autoimmune and inflammatory conditions and could be effective in numerous conditions.

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