PP-167

The Novel Aldehyde Trap, ADX-102, Reduces Inflammation-Mediated Lung Infiltrate in a Mouse Model of LPS-Induced Acute Lung Injury

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Objective

This study was conducted to evaluate the activity of the novel aldehyde trap, ADX-102, in LPS-induced acute lung injury.

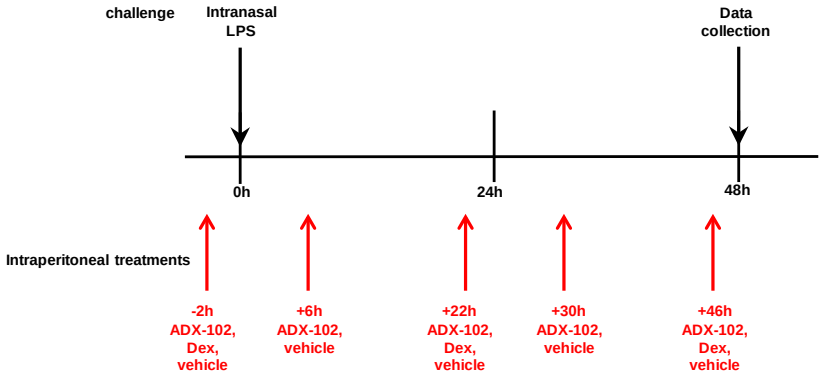
Case

Clinical and preclinical data implicate aldehydes as pro-inflammatory mediators. ADX-102, a novel aldehyde sequestering agent, has been shown to inhibit the NFκB pathway and fibrotic changes in cardiac myofibroblasts; modulate inflammatory responses in animal models of inflammation; and diminish inflammation in Phase 2 clinical trials in noninfectious anterior uveitis and allergic conjunctivitis. In addition, increased malondialdehyde levels have been observed in lipopolysaccharide (LPS)-induced models of acute respiratory distress syndrome (ARDS). Thus, ADX-102 was tested in a mouse model of LPS-induced lung injury, which may mimic conditions observed in human ARDS.

Materials and Methods

Mice (n = 8 per group) were challenged intranasally with 10 µg of LPS (derived from *Pseudomonas aeruginosa*) to induce pulmonary inflammation, or saline as a control. As shown in the schedule outlined in Figure 1, animals were administered intraperitoneal ADX-102, vehicle, or dexamethasone. After the last treatment, animals were anaesthetized, blood was collected, and lung function was measured. Animals were then sacrificed, bronchoalveolar lavage (BAL) was performed, and BAL fluid (BALF) collected and analyzed.

Figure 1



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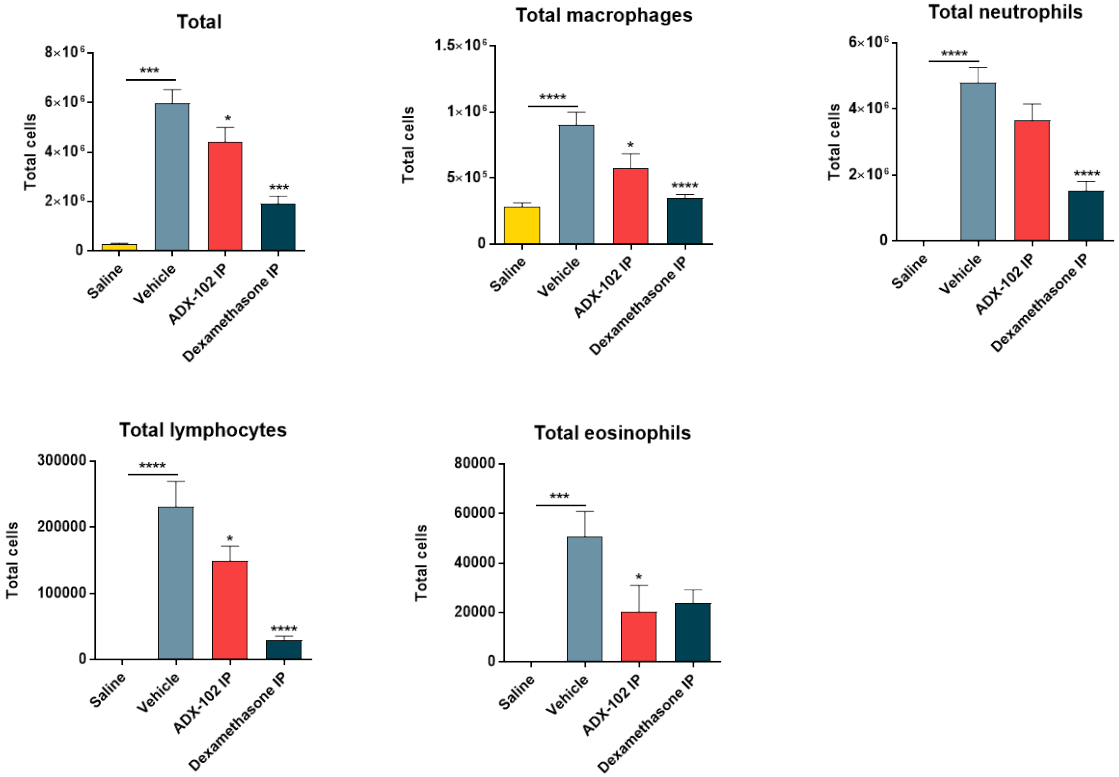
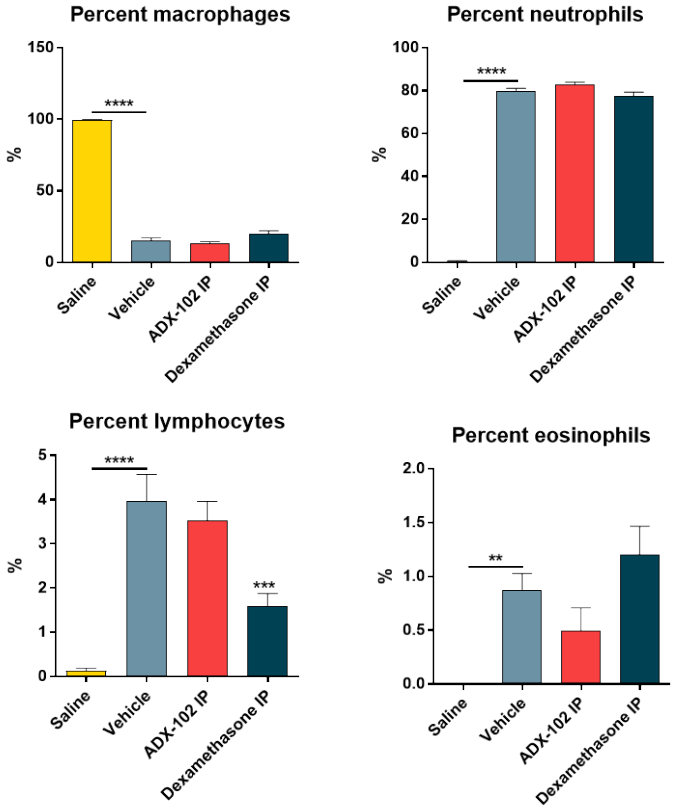
Results

LPS challenge perturbed the pressure-volume relationship in the lung, showing a shift down and to the right, indicating decreased volume associated with increased pressure, as compared to the saline control group, which is indicative of increased lung stiffness. LPS challenge also led to increases in total cells, macrophages, neutrophils, lymphocytes and eosinophils; total protein; and pro-inflammatory cytokines and chemokines in the BALF.

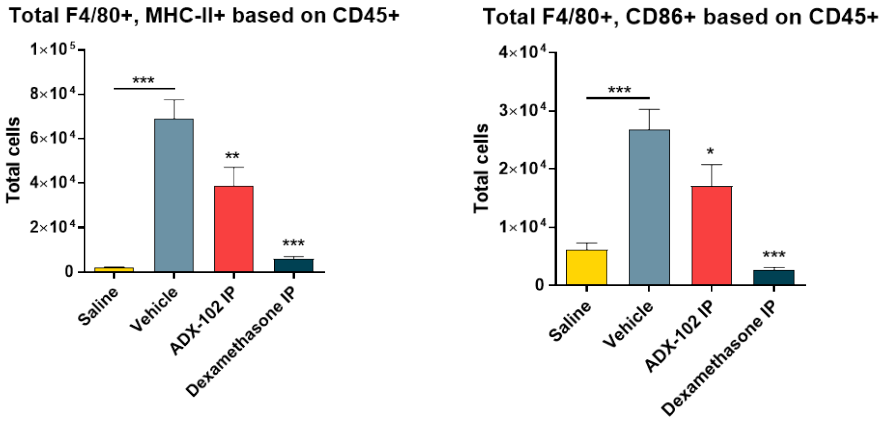
ADX-102 treatment resulted in statistically significant reductions in total cells, macrophages, lymphocytes and eosinophils in BALF, with little or no effect on relative proportions of the cell types (Figures 2a and 2b). In addition, FACS analysis showed significant reductions in populations of activated macrophages, relative to vehicle control (Figure 3a), although ADX-102 did not alter the level of activation of each cell (Figure 3b).



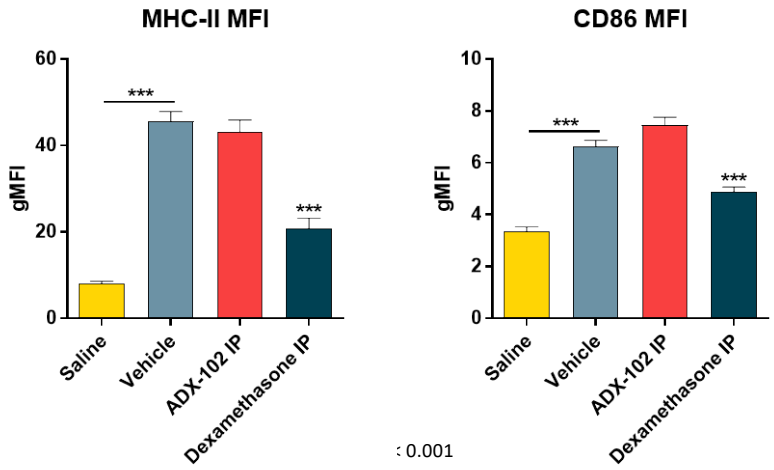
01; **** $p < 0.0001$



* p < 0.05; *** p < 0.001; ****p < 0.0001

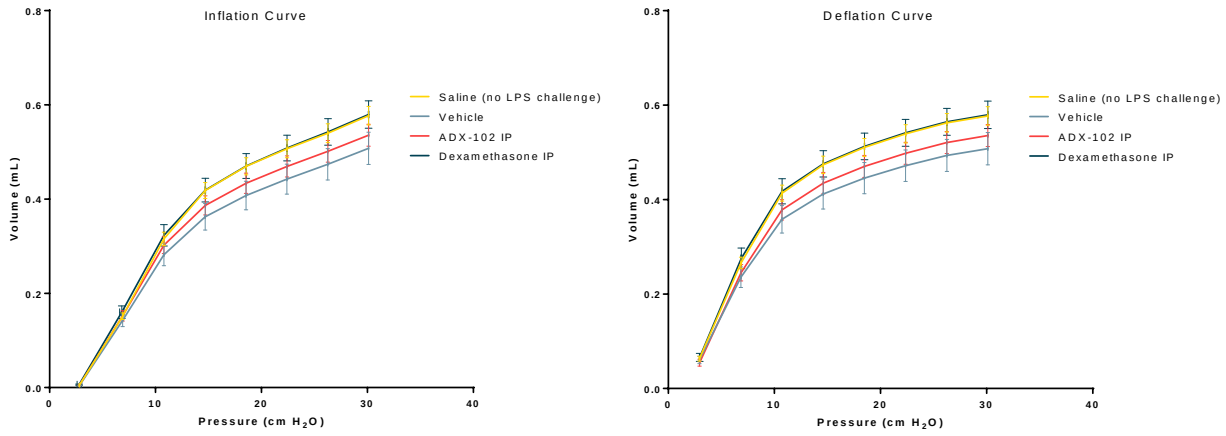


1; *** p < 0.001

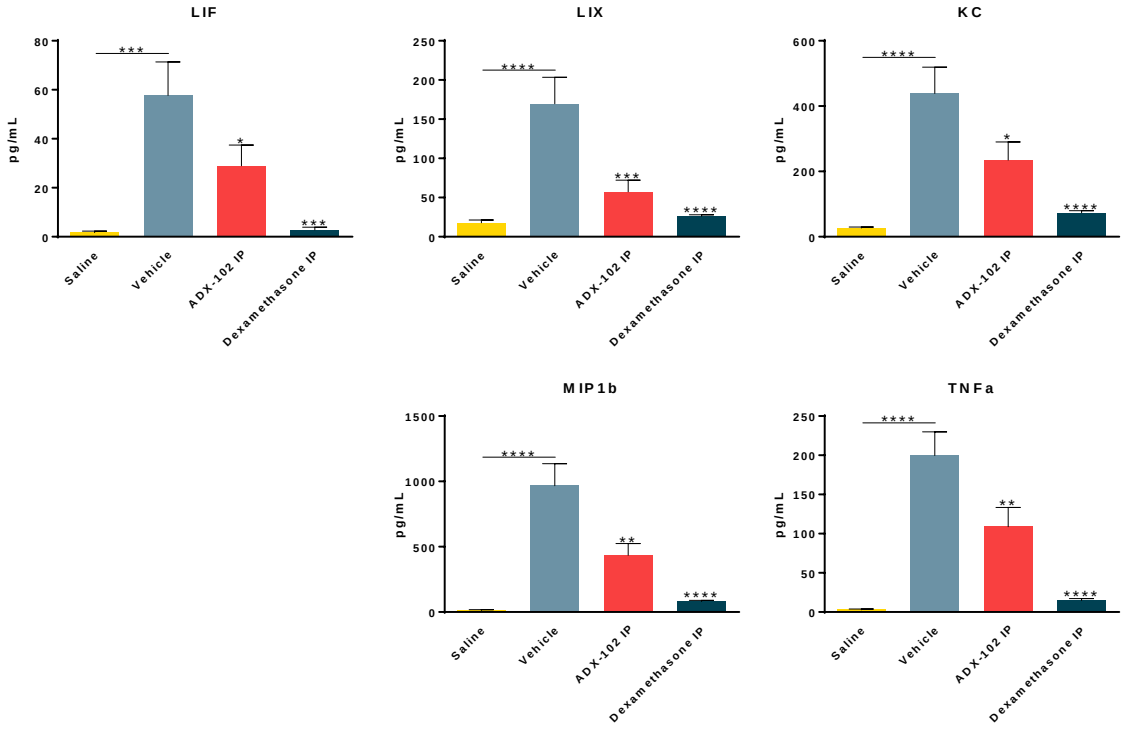


: 0.001

In lung mechanics tests, LPS-challenged mice treated with ADX-102 showed a curve shift up and to the left relative to the vehicle, indicating substantially reduced lung stiffness and improved lung compliance and elastance, although these measures were not statistically significant (Figure 4).



ADX-102 treatment also significantly decreased LPS-induced increases in several pro-inflammatory cytokines (IFNγ and IL-1α, IL-12p40, IL12p70 and IL-17; Figure 5a) and chemokines (LIF, LIX, KC, MIP-1α, MIP-1β and TNFα; Figure 5b). There was also a statistically significant decrease in total protein in BALF (Figure 6).



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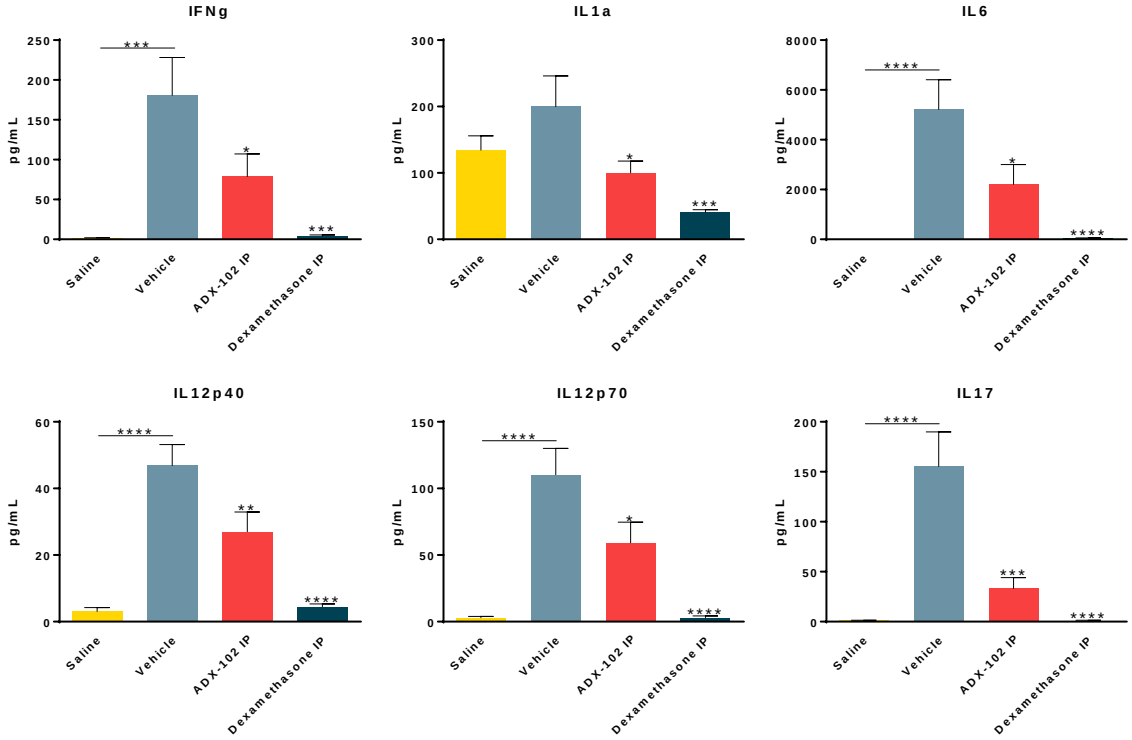
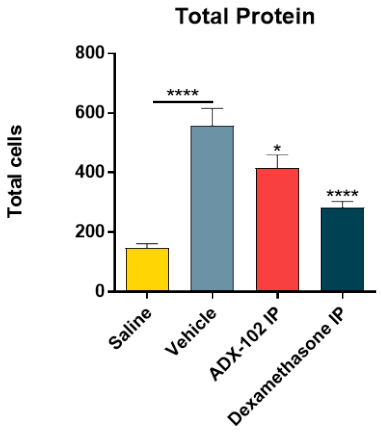


< 0.0001

Figure 6



Discussion

Treatment of LPS-challenged animals with ADX-102 statistically significantly reduced LPS-induced increases in total cells, macrophages, lymphocytes and eosinophils in BALF. Although neutrophils were also reduced, statistical significance was not reached in that cell population, perhaps because these cells were the most numerous and abundant cell type that migrated into the lung tissue. However, ADX-102 treatment did not affect the relative proportions of infiltrating cells, compared to vehicle, whereas dexamethasone significantly reduced the proportion of lymphocytes, suggesting ADX-102 and corticosteroids may act through different mechanisms.

Consistent with the cell count data, FACS analysis showed significant reductions in the amounts of both activated macrophage populations [(F4/80+, MHC-II+) and (F4/80+, CD86+)], as compared to the vehicle group, with ADX-102 treatment. However, ADX-102 did not significantly alter the level of activation of each cell, as measured by MFI for MHC-II and CD86, as compared to the vehicle group, suggesting that there were no phenotypic changes in the macrophage population with ADX-102 treatment. In contrast, dexamethasone significantly reduced MFI for both MHC-II and CD86, again suggesting that ADX-102 may affect different pathway(s) than corticosteroids.

Lung compliance and elastance also improved with ADX-102 treatment, which is suggestive of beneficial effects on lung mechanics. Although these measures were not statistically significant, the window for observing an effect of ADX-102 (or dexamethasone) was minimal, due to the small changes in lung function induced by LPS challenge. In addition, the dose and schedule of ADX-102 treatment has not been optimized.

ADX-102 treatment also resulted in statistically significant reductions in IFNγ, IL-6, IL-12p40, IL-12p70, IL-17, LIF, LIX, KC, MIP-1α, MIP-1β, and TNFα in BALF. Many of these have been shown to be upregulated in animal models of lung injury, and some have been linked to the NFκB signaling pathway. Treatment with dexamethasone resulted in a different pattern of reductions in cytokine and chemokine levels, further supporting the notion that ADX-102 and corticosteroids do not act through identical pathways.

The decrease in total protein in BALF following ADX-102 treatment is consistent with reduced infiltration of inflammatory cells and the presence of chemokines and cytokines, suggesting that ADX-102 may act

via a novel anti-inflammatory mechanism that could involve reduced vascular permeability in lung and/or diminished chemotaxis.

Conclusions

The data show that ADX-102 can significantly reduce inflammation in LPS-induced lung injury via a mechanism that differs from corticosteroids, and build on existing evidence that aldehyde sequestration represents a novel anti-inflammatory therapeutic approach.