

Olanzapine increases severity of pulmonary *Staphylococcus aureus* infection in mice through reduced neutrophil recruitment and activation

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Research Article

Keywords:

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11234457/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

Second generation antipsychotics (SGAs) are front-line treatments for schizophrenia and related psychoses. Recent evidence has linked SGA use with an increased risk of pneumonia-related deaths. However, the mechanisms underlying this increased susceptibility remain unclear. In this study we evaluated the role of the SGA olanzapine on the immune response to infection with the clinically relevant strain of *Staphylococcus aureus* USA300.

Methods

In vitro studies: Isolated whole blood obtained from healthy volunteers was stimulated with USA300 in the presence and absence of 6, 20 or 60ng/ml olanzapine and phenotyped using flow cytometry. **In vivo studies:** C57Bl/6 mice were treated with 2.2mg/g olanzapine via oral gavage for 16 days. On day 14, mice received intrapulmonary USA300 (1×10^6 cfu/mouse) in the presence or absence of prior lung injury induced by repeated aspiration with 2mg/kg Porcine Pancreatic Elastase (PPE) on days 4, 8 and 12 (a two-hit model to recapitulate chronic lung injury). A subgroup of animals also received daily therapy with the inhaled corticosteroid budesonide. 4, 24, and 48 hours post infection, animals were euthanised and microbiological and inflammatory parameters were quantified. **Pharmacoepidemiology of deaths in patients prescribed olanzapine:** We evaluated the association of pneumonia-related deaths, olanzapine therapy, respiratory comorbidities and inhaled corticosteroid (ICS) prescribing using the UK-based National Programme on Substance Use Mortality (NPSUM) database of drug-related deaths.

Results

In *in vitro* stimulated whole blood, olanzapine was associated with reduced expression of granulocyte CD88, IL-1 β and IL-6, and increased CXCR4. Olanzapine treatment significantly reduced neutrophil recruitment and activation in immunocompetent mice, although not associated with changes in bacterial burden. In the two-hit murine infection model, olanzapine treatment significantly reduced pulmonary TNF- α and neutrophil elastase, which was associated with increased pulmonary, kidney and splenic bacterial load, and a systemic inflammatory response. Combination therapy with the corticosteroid budesonide further impaired bacterial clearance. Pharmacoepidemiological analysis revealed an increased risk of pneumonia-related deaths in patients co-prescribed olanzapine and inhaled ICSs – used as a proxy marker of co-morbid lung disease – vs patients prescribed olanzapine in the absence of an ICS.

Conclusion

Olanzapine suppresses neutrophil responses, increasing disease severity from lung infection in mice with prior lung injury. This was exacerbated by concomitant administration of the ICS, budesonide. Our data suggests that patients on olanzapine may have a compromised immune response to bacterial pneumonia which may contribute to clinical observations that patients taking regular olanzapine have an increased incidence of pneumonia, particularly if they are receiving an ICS for a co-morbidity.

Background

Second generation antipsychotics (SGAs) such as clozapine, olanzapine, quetiapine and risperidone are the most effective acute and long-term treatments for schizophrenia and related psychosis (1). Whilst known adverse effects of SGAs include weight gain, lethargy, sedation and headache (2), other reports have highlighted an association between SGA use and pneumonia (3, 4). Clozapine has a well-recognised association with neutropenia increasing susceptibility to bacterial infections, resulting in limited clozapine prescribing. However, there is some evidence that other SGAs, including the structurally similar olanzapine and quetiapine, may also induce neutropenia (5–9). Research by our own group using the National Programme on Substance Use Mortality (NPSUM) revealed that olanzapine and quetiapine also increased the risk of pneumonia-related deaths with adjusted odds ratios (AORs) of 1.49 and 1.44 respectively (10) suggesting a link between the two SGAs and pneumonia. These findings therefore raise the possibility that certain SGAs can impair the host immune system to increase susceptibility to severe infection.

Whilst SGAs are not primarily immunomodulatory drugs, recent mechanistic studies and *in vivo* models have demonstrated that chronic olanzapine therapy can alter the expression of inflammatory cytokines including IL-6, IL-1 β , IL-2, IL-10 and TNF- α (11–17). Other immunosuppressive mechanisms include off target effects of SGAs on the platelet-activating factor receptor (PTAFR) and thromboxane A2 receptor (18). These receptors mediate capillary permeability at the alveolar level, leading to alveolar oedema which may increase susceptibility to pneumonia (19).

The biological mechanisms underlying this susceptibility to pneumonia in patients on chronic SGAs remains unclear. Given their widespread use in the treatment of schizophrenia and psychosis, there is a clear need to understand this interaction in more detail to improve the pharmacovigilance of SGA use. In this study, we have therefore evaluated the impact of olanzapine on immune cell function in response to bacterial stimulation using the community-acquired strain of the Gram-positive bacteria *Staphylococcus aureus* (USA300) both *in vitro* using healthy volunteer human blood and in *in vivo* murine models of pulmonary infection.

Materials and Methods

In vitro bacterial culture conditions and maintenance for bacterial infection.

The methicillin resistant community acquired *S. aureus* strain USA300 (20) was donated by Dr Alessandra Bragonzi from the Infection and Cystic Fibrosis Unit, San Raffaele Scientific Institute, Milan,

Italy. Following collection and culture, bacteria were stored at -80°C. For all studies, a loopful of USA300 was taken from the frozen stock cultures and streaked onto a trypticase soy agar (TSA) plate and incubated at 37°C overnight. 2- colonies were then inoculated into 20 ml trypticase soy broth (TSB) and incubated for 16 hours at 37°C under shaking conditions (120rpm). Post incubation, the overnight culture was diluted by placing 2 OD (Optical Density; absorbance at 600nm) of bacterial culture (approximately 1×10^9 cfu) into 20ml of fresh TSB and incubated for 3–4 hours to reach log phase of growth. Once at log phase, bacterial cultures were centrifuged at 2700 g for 20 minutes at 4°C and suspended in sterile PBS. Cultures were then diluted to a working concentration of 2×10^7 cfu/ml (OD 0.1).

In vitro healthy volunteer blood stimulation

Previous work has suggested that SGA therapy can alter immune cell activation and cytokine release. We therefore first examined whether olanzapine therapy affected immune cell activation in healthy human peripheral blood.

Ethical approval

for healthy volunteer work was obtained from the University College London Research Ethics Committee (reference 19181/001). We adapted previously published protocols (**PMID: 38655251 & 42376746**) and stimulated healthy volunteer whole blood or peripheral blood mononuclear cells (PBMC) with USA300 (1×10^7 cfu/ml) and three concentrations of olanzapine (6, 20, and 60 ng/ml) for 15 minutes, representing the range of patient serum concentrations based on pharmacokinetic data (**PMID: 19440083**)).

The following flow cytometry panels were used to assess granulocyte, classical monocyte, CD4⁺ and CD8⁺ lymphocyte immunophenotypes:

- Panel 1: Granulocyte phagocytosis – pHRodo, CD16, CD66b, and DCFDA
- Panel 2: Granulocyte cell markers and cytokines – CD16, CD24, CD66b, CD86, CD88, CXCR4, IL-1 β , and IL-6.
- Panel 3: Monocyte phagocytosis – pHRodo, CD14, CD16, HLA-DR, and DCFDA
- Panel 4: Monocyte cell markers and cytokines – CD14, CD16, HLA-DR, CCR2, CXCR4, CD80, CD86, PD-L1, IL-1 β , IL-6, IL-10, and TNF- α .
- Panel 5: Lymphocyte proliferation and cytokines – CellTrace, CD3, CD4, CD8, CD19, IL-2, IL-10, and IFN- γ .
- Panel 6: Lymphocyte cell markers – CD3, CD4, CD8, CD19, CD25 (IL-2R), CD28, CD127 (IL-7R), CD152 (CTLA-4), CD274 (PD-L1), and CD279 (PD-1).

Full details on products, concentrations and methods are available in the **Supplemental Methods**.

In vivo murine model

Given the role played by neutrophils in the innate immune response to bacterial infection, we evaluated the impact of olanzapine therapy on the host immune response in a murine model of acute pulmonary infection with USA300. Animals were treated with 2.2mg/kg/day olanzapine or vehicle for 14 days prior to infection with 1×10^6 cfu/mouse USA300. The olanzapine dose used in this study is the murine equivalent dose of olanzapine used clinically. Animals were then culled 4-, 24- and 48-hours post infection by terminal anaesthesia with 25% w/v urethane and parameters of pulmonary inflammation and bacterial burden assessed.

Animals

All animal experiments were performed in accordance with the Animals (Scientific Procedures) Act 1986 with 2012 amendments (PP1060882), with local ethical approval from the KCL Animal Welfare and Ethical Approval Board (AWERB). All studies are reported in compliance with ARRIVE guidelines. Animal numbers were determined by power calculations and internal laboratory experience. Male and Female mice aged 8–10 weeks were sourced from a commercial breeder (Charles River, UK). Mice were housed in individually ventilated cages under standard conditions of $22 \pm 2^\circ\text{C}$ with a 12:12 light:dark cycle. Mice had access to chow RM1(E) diet (Special Diets Service) and water *ad libitum* throughout and provided with wood shavings, shredded paper and cardboard tubes for environmental enrichment. All animals were provided with a minimum acclimatisation period of 7 days upon arrival at KCL before the commencement of the study.

Acute pulmonary infection with USA300.

To investigate the impact of the olanzapine on the host immune response to pulmonary infection, mice were dosed with 2.2mg/kg/day olanzapine or vehicle control (1% DMSO in PBS) once daily via oral gavage (*p.o.*) for 16 days. 14 days post-onset of olanzapine therapy, animals were anaesthetised under inhaled isoflurane anaesthesia and infected with 50µl 2×10^7 cfu/ml USA300 via intratracheal (*i.t.*) administration, delivering a final inoculum of 1×10^6 cfu/mouse. Animals were monitored at regular intervals for signs of pain and distress, and body weight measured daily.

To fully model the clinical scenario where a schizophrenia patient prescribed olanzapine suffers from a chronic respiratory comorbidity resulting in the requirement for concomitant therapy with an ICS, our final set of experiments was to evaluate the impact of combining olanzapine treatment with the ICS, budesonide, in our two-hit model of pulmonary infection with USA300 as previously described (21), given the known additional immunosuppressive effects of inhaled corticosteroids. Mice were challenged with 2mg/kg porcine pancreatic elastase via intranasal (*i.n.*) administration on days 4, 7 and 12 of olanzapine dosing. In further studies, to assess the impact of ICS treatment, animals were treated with 3.3mg/kg/day budesonide (*i.n.*) daily concomitantly with olanzapine therapy.

Quantification of inflammatory cell recruitment

4-, 24- and 48-hours post infection with *S. aureus* USA300, animals were terminally euthanised using 25% w/v urethane via intraperitoneal (*i.p.*) injection. At each time-point peripheral blood was collected by cardiac puncture into syringes preloaded with acid citrate dextrose (ACD) at a ratio of 1:9 ACD:Blood. After blood collection, a bronchoalveolar lavage (BAL) was performed via the insertion of a 22-gauge cannula into the trachea and flushed 3 times with sterile PBS. Total cell counts were enumerated by adding BAL fluid to Tuerk's nuclear stain in a 1:1 ratio and quantified using an improved Neubauer chamber and a bright field microscope under a 20X objective (Leica DM2000 LED). Cytospin slides were prepared and 100µl BAL fluid added into a cytopsin funnel and centrifuged at 1000 rpm for 5 minutes under medium acceleration (Shandon, UK). Slides were then stained using Diff-Quick and the percentage of inflammatory cells calculated over a representative portion of the slide.

Quantification of pulmonary and systemic bacterial load

After BAL, at each time point, lungs, spleen and kidneys were aseptically removed and homogenised in 2ml sterile PBS. Tissue homogenates were then serially diluted 1:10 in sterile PBS and plated on TSA plates which were then incubated at 37°C overnight under static conditions and bacterial colony forming units (CFUs) enumerated.

Quantification of soluble inflammatory markers using ELISA

At each timepoint, BAL fluid and peripheral blood was centrifuged at 4000 g for 10 minutes at 4°C and BAL supernatant and blood plasma stored at -80°C for subsequent cytokine analysis. Total levels of soluble inflammatory markers including TNFα, Neutrophil Elastase (NE) and C-reactive protein (CRP), were measured in BAL fluid and plasma by ELISA according to the manufacturer's (R&D Systems) protocols.

The National Programme on Substance Use Mortality (NPSUM)

In this study we evaluated the association of pneumonia-related deaths, olanzapine therapy, respiratory comorbidities and ICS prescribing using the UK-based drug-related deaths database, the NPSUM. The King's College London Biomedical and Health Sciences, Dentistry, Medicine and Natural and Mathematical Sciences Research Ethics Sub-Committee (BDM-RESC) re-confirmed in August 2026, that NPSUM does not require BDM-RESC review as all subjects are deceased.

Case Identification

Cases reported by 1st March 2025 where olanzapine had been prescribed to decedents were extracted from the NPSUM database to evaluate the rate of ICS co-prescribing. ICS prescribing was used as a proxy measure of co-morbid lung disease. Smoking status of decedents is not routinely reported to the NPSUM.

Analysis was carried out as previously described (10). Odds ratios (ORs) were calculated to test if the incidence of pneumonia-related death was worsened in patients co-prescribed olanzapine and ICSs vs prescribing of olanzapine without an accompanying ICS prescription.

Data Presentation and Analysis

Data from *in vitro* and *in vivo* studies are expressed as mean ($\pm$ SEM). Data were tested for normality using the Shapiro-Wilks normality test in GraphPad Prism (Version 10.6.0). Data that passed normality testing were analysed using a one-way ANOVA with Sidaks multiple comparison post-test, or students t-test when only 2 groups were analysed. Data was plotted using GraphPad prism. A P value < 0.05 was considered significant.

NPSUM data analysis and statistical tests (ORs) were performed using IBM® SPSS™ Statistics for Windows version 31 (IBM Corp, New York, USA).

Results

Olanzapine treatment reduces granulocyte activation following stimulation with USA300 *in vitro*

In granulocytes, there were no differences in CD16, CD24, CD66b or CD86 expression observed following olanzapine therapy (**Supplementary Fig. 1A-D**). However, we observed a trend for a concentration-dependent reduction in CD88 expression following olanzapine treatment, although this did not reach statistical significance (**Supplementary Fig. 1E**). Additionally, we observed a non-significant trend for an olanzapine mediated increase in CXCR4 (**Supplementary Fig. 1F**). No differences were detected for either DCDFA (**Supp Fig. 1G**) or pHRodo (**Supp Fig. 1H**) following olanzapine therapy as a measure of reactive oxygen species (ROS) production and phagocytosis respectively. We also observed a non-significant trend for a reduction in intracellular cytokines IL-1 β (**Supp Fig. 1I**) and IL-6 (**Supp Fig. 1J**) respectively. On analysis of monocyte activation, no changes in surface expression of HLA-DR, CCR2, CD80, CD86, CXCR4 or PD-L1 were observed (**Supp Fig. 2A-F**). However, infection induced reductions in both DCDFA (**Supp Fig. 2G**) and pHRodo (**Supp Fig. 2H**) were significantly reversed following olanzapine treatment. However, no changes in intracellular IL-1 β or IL-6 were observed (**Supp Fig. 2I & J**). Finally, CD127 was significantly reduced in both CD4⁺ and CD8⁺ T cells following stimulation with USA300, which was reversed with olanzapine treatment (**Supp Fig. 3D & 4D**). No other differences in cell surface expression markers or intracellular cytokines were detected between olanzapine or vehicle treated controls for either CD4⁺ or CD8⁺ T Cells respectively (**Supplementary Figs. 3 & 4**).

Taken together, the analysis of human immune cells isolated from healthy peripheral blood, pretreated with olanzapine prior to stimulation with USA300, revealed several alterations in innate immune phenotypes and cytokine production in granulocytes and monocytes.

Olanzapine impairs pulmonary inflammation to pulmonary infection with USA300 in a murine model

Infection with 1×10^6 cfu/mouse induced localised resolving pulmonary infection at all time points compared to sham infected controls with peak infection 4 hours following inoculation (Sham: 0 ± 0 cfu/lung; 4hrs: 6.53 ± 0.22 log cfu/lung; 24hrs: 3.14 ± 0.13 cfu/lung; 48hrs: 2.16 ± 0.23 log cfu/lung, Fig. 1A). This was associated with pulmonary leukocyte recruitment (26.63 ± 3.97 cells/ml at 4 hours, 62.42 ± 17.21 cells/ml at 24 hours; and 19.61 ± 4.07 cells/ml at 48 hours, Fig. 1B), which was reflected by a concurrent increase in both neutrophils (4hrs: 8.38×10^4 cells/ml; 24hrs: $8.61 \pm 5.00 \times 10^4$ cells/ml; 48hrs: $9.41 \pm 1.96 \times 10^4$ cells/ml, Fig. 1C) and mononuclear cells (4hrs: $15.25 \pm 4.41 \times 10^4$ cells/ml; 24hrs: $19.62 \pm 6.85 \times 10^4$ cells/ml; 48hrs: $10.20 \pm 2.97 \times 10^4$ cells/ml, Fig. 1D). Treatment with 2.2mg/kg olanzapine daily had no effect on bacterial load at any time point (Fig. 1A). Furthermore, no significant differences were observed between vehicle or olanzapine treated animals for either total pulmonary leukocyte (Fig. 1B) or monocyte recruitment (Fig. 1D). However, 4 hours post-infection, olanzapine treatment significantly reduced pulmonary neutrophil recruitment compared to vehicle controls (Vehicle: $8.38 \pm 0.79 \times 10^4$ cells/ml vs OLZ: $3.63 \pm 0.73 \times 10^4$ cells/ml, $P < 0.001$, Fig. 1C). This was also reflected by a significant reduction in neutrophil activation as measured by neutrophil elastase levels in BAL fluid (Veh: 454 ± 99 pg/ml vs OLZ: 222 ± 36 pg/ml, $P < 0.05$, Fig. 1E) and in the levels of TNF- α (Veh: 3987 ± 632 pg/ml vs OLZ: 2099 ± 627 pg/ml, $P < 0.05$, Fig. 1F).

NPSUM Analysis

Initial studies using immunocompetent healthy mice demonstrated a significant impairment of pulmonary neutrophil recruitment and activation in response to olanzapine therapy. However, this did not impact either pulmonary bacterial load or the development of systemic infection. We therefore hypothesised that a subpopulation of patients with underlying lung disease/injury and prescribed chronic olanzapine therapy may be more prone to infections than others. ICS prescribing was used as a proxy measure of co-morbid inflammatory lung disease.

There were 3,218 decedents reported to the NPSUM by 1st March 2025 who were in receipt of an active olanzapine prescription at the time of their death, of which 96 had been co-prescribed an ICS and 3,122 who had not. Pneumonia was cited as a cause of death in 10 patients who were co-prescribed olanzapine and an ICS and 147 who had been prescribed olanzapine in the absence of an ICS. As incidence of pneumonia-related death in the co-prescribed population was therefore 2.35 times higher than in those prescribed olanzapine in the absence of an ICS (95% CIs 1.20–4.62, $p < 0.01$), we next evaluated the impact of olanzapine therapy in a two-hit murine model of pulmonary infection. This incorporates prior elastase-mediated lung injury as a model of chronic respiratory disease prior to pulmonary infection with USA300, as described previously (21).

Olanzapine significantly impairs the immune response to pulmonary infection in animals with underlying lung injury

Infection with 1×10^6 cfu/mouse USA300 following repeated elastase challenge resulted in significant pulmonary infection at all timepoints which peaked at 4 hours post infection (4hrs: 4.91 ± 0.08 log cfu/lung; 24hrs: 3.77 ± 0.08 log cfu/lung; 48hrs: 2.89 ± 0.07 log cfu/lung, Fig. 2A), with bacterial numbers in systemic organs such as the spleen (Fig. 2B) and kidney (Fig. 2C) remaining below the limit of detection (< 1.5 log cfu/organ). This pulmonary infection was associated with significant influx of inflammatory leukocytes (4hrs: $29.08 \pm 4.04 \times 10^4$ cells/ml; 24hrs: $28.61 \pm 5.51 \times 10^4$ cells/ml; 48hrs: $35.17 \pm 5.66 \times 10^4$ cells/ml, Fig. 2D) characterised by both pulmonary neutrophilia (4hrs: $10.37 \pm 1.82 \times 10^4$ cells/ml, 24hrs: $6.55 \pm 1.85 \times 10^4$ cells/ml, 48hrs: $12.65 \pm 3.07 \times 10^4$ cells/ml, Fig. 2E) and monocyte recruitment (4hrs: $19.45 \pm 3.06 \times 10^4$ cells/ml, 24hrs: $21.12 \pm 4.03 \times 10^4$ cells/ml, 48hrs: $22.52 \pm 3.36 \times 10^4$ cells/ml, Fig. 2F).

Following daily olanzapine therapy, significant increases in pulmonary bacterial load were seen compared to vehicle controls at both 4 (Veh: 4.91 ± 0.08 vs OLZ: 5.60 ± 0.10 log cfu/lung, $P < 0.001$ and 24hrs (Veh: 3.77 ± 0.08 vs OLZ: 4.05 ± 0.08 log cfu/lung, $P < 0.05$) post infection (Fig. 2A). This was also associated with significant increases in bacterial load detected in both the spleen (Veh: 0.78 ± 0.37 vs OLZ: 2.38 ± 0.21 log cfu/spleen, $P < 0.001$, Fig. 2B) and kidney (Veh: 1.08 ± 0.46 vs OLZ: 2.78 ± 0.36 log cfu/kidney, $P < 0.01$) at 4 hours post infection consistent with the infection spreading from the lung systemically. This observation was accompanied by reductions in pulmonary neutrophil recruitment, with a non-significant reduction observed at 4 hours post infection (Veh: $10.37 \pm 1.82 \times 10^4$ cells/ml vs OLZ: $5.00 \pm 1.20 \times 10^4$ cells/ml), and a significant reduction at 48 hours post infection (Veh: $12.65 \pm 3.07 \times 10^4$ cells/ml vs OLZ: $4.36 \pm 1.40 \times 10^4$ cells/ml, $P < 0.05$) (Fig. 2D). No changes in mononuclear cells were detected (Fig. 2E). We also observed reduced pulmonary levels of (BAL Fluid) TNF- α (24hrs: $P < 0.05$), Neutrophil Elastase and CRP (4hrs: $P < 0.001$) (Figs. 3A-C). However, whilst local inflammatory responses were observed to be suppressed, markers of systemic inflammation were significantly elevated 24 hours post infection as measured by plasma levels of TNF- α ($P < 0.05$), Neutrophil Elastase ($P < 0.05$) and CRP ($P < 0.05$) (Figs. 3C-E).

Combined treatment with olanzapine and budesonide delays bacterial clearance in a two-hit model of pulmonary infection with USA300.

Budesonide therapy was associated with a significant elevation in pulmonary bacterial load at 48 hours post infection (OLZ + Veh: 3.04 ± 0.14 log cfu/lung vs OLZ + BUD: 3.53 ± 0.12 log cfu/lung, $P < 0.05$, Fig. 4A). Budesonide therapy in addition to olanzapine therapy had no additional impact on the degree of bacterial load in either the spleen (Fig. 4B) or kidney (Fig. 4C), or on pulmonary leukocyte recruitment (Figs. 4D-F).

Discussion

SGAs such as olanzapine are one of the most commonly prescribed antipsychotics. However, there is increasing evidence linking chronic SGA prescription with increased risks of pneumonia-related deaths (10, 22, 23). Whilst agranulocytosis has been recognised, albeit rare (24), the mechanisms behind this association are unclear, research has suggested that this may include effects of these drugs on immune cell function (11–17). In this study we identified that the SGA olanzapine significantly reduced neutrophil recruitment and activation in a model of lung infection, but that in immunocompetent mice, this was not sufficient to alter the progress of the respiratory infection. However, in animals subjected to prior lung injury to model chronic respiratory diseases such as COPD, and/or receiving the ICS budesonide, treatment with olanzapine then had a significant impact on neutrophil function leading to an increased pulmonary bacterial load and the development of systemic infection and associated inflammation. These findings were consistent with analysis of the NPSUM that revealed that patients prescribed ICS alongside olanzapine had a higher risk of pneumonia-related death.

Preliminary *in vitro* studies were inconclusive but highlighted a trend for a reduction in the cell surface granulocyte CD88 (responsible for chemotaxis and ROS production) and intracellular cytokines IL-1 β and IL-6 induced by USA300 stimulation in the presence of olanzapine consistent with previous literature, potentially indicating an olanzapine-mediated inhibition of neutrophil activation, independent of granulopoiesis (16, 25).

Following infection with *S. aureus* USA300 in immunocompetent mice, we observed a significant reduction in both pulmonary neutrophil recruitment and activation (as measured by pulmonary neutrophil elastase release) following olanzapine therapy. Anecdotal evidence has reported that similar to clozapine, olanzapine therapy can also induce neutropenia in patients (5–9). However, total systemic leukocyte numbers were unchanged in our study suggesting that olanzapine directly interferes with neutrophil recruitment and activation following bacterial infection rather than influencing neutrophil production. Furthermore, our studies also revealed a significant reduction in the release of proinflammatory cytokines including TNF- α . As TNF- α is one of the earliest pro-inflammatory cytokines released from alveolar macrophages and epithelial cells following pathogen invasion, this suggests a potential wider suppression of innate immunity, consistent with previous studies which have shown that olanzapine can reduce the release of pro-inflammatory cytokines including TNF- α , IL-6, IFN- γ , and IL-12 from inflammatory cells (26–29). Therefore, whilst we observed significant impacts on neutrophil recruitment and activation, these data would also suggest a wider impact of olanzapine on the innate immune response within the respiratory tract. The combination of impaired localised inflammation alongside an increase in both systemic bacterial load and inflammatory markers is consistent with enhanced severity of the infection in animals treated with olanzapine compared to vehicle controls.

Whilst studies in immunocompetent mice revealed an olanzapine mediated reduction in innate immune responses to pulmonary infection, these modest changes did not translate to an increased infection severity. We postulated that a high-risk sub-population of patients prescribed olanzapine exhibit this increased susceptibility to pneumonia-related mortality. It is widely known that 70–80% of patients with schizophrenia are heavy smokers, with 30–40% diagnosed with COPD (30, 31). Chronic inflammation

associated with COPD results in localised dysfunctional immune responses characterised by functional impairment to inflammatory cells including alveolar macrophages, airway epithelial cells and neutrophils underpinning progressive structural damage, and impaired pathogen clearance (32). ICS remain highly prescribed in COPD patients alongside bronchodilators, and further analysis of the NPSUM revealed that patients receiving ICS treatment demonstrated a greater susceptibility to pneumonia-related death. We therefore evaluated the effect of olanzapine using a two-hit model where animals had an underlying lung injury following treatment with repeated elastase challenge to model lung injury found in patients with COPD prior to infection (21).

We and others have previously reported that repeated challenge with PPE induces a lung phenotype consistent with the COPD lung characterised by pulmonary neutrophilia, alveolar damage, and mucus hypersecretion (21, 33, 34). Significantly this model demonstrates reduced pro-inflammatory cytokine release such as TNF- α , IL-6, MCP-1 and mouse KC (the functional murine homolog of human IL-8) in response to bacterial infection highlighting impaired innate immune function which led to impaired pathogen clearance (21). In the current study, olanzapine therapy further suppressed infection-induced pulmonary neutrophil recruitment and activation, and pulmonary pro-inflammatory cytokine release (TNF- α and CRP) compared to vehicle controls, which was associated with increased bacterial load 4 and 24 hours post infection. Notably, olanzapine mediated exacerbation of localised immune suppression was also associated with the induction of a systemic immune response and disseminated infection, consistent with poorer clinical outcomes and increased mortality as observed in the NPSUM analysis (10).

These studies clinically suggest a scenario whereby olanzapine mediated immune suppression on its own is insufficient to increase the risk of pneumonia, yet when combined with prior neutrophilic lung damage, the response to infection is significantly impaired. In the NPSUM analysis we used ICS prescription as a marker of co-morbid respiratory disease, however ICS are well known for their immunosuppressive activity. We therefore conducted final studies where ICS therapy was considered as a treatment-related factor in addition to olanzapine in the 2-hit model through intranasal administration of the corticosteroid budesonide. Whilst budesonide is known for its anti-inflammatory functions, we did not see any further reductions in neutrophil recruitment/activation with the addition of budesonide treatment, nor did we see any further increase in disseminated infection. However, we did observe elevated pulmonary bacterial load at 48 hours, indicating that the combination of both olanzapine and budesonide in the diseased lung further impairs bacterial clearance. These results are consistent with both the NPSUM analysis and widespread reports that long-term ICS therapy is associated with increased pneumonia risk through impaired bacterial clearance (35–37).

Limitations of the study

Whilst this study demonstrates a clear role for olanzapine mediated impairment of the immune response, this study possesses a number of limitations. *In vitro* experiments on healthy human peripheral blood were restricted to stimulation with heat-killed USA300. Whilst heat-killed bacteria retain

an ability to stimulate surface expressed PRRs, there may be reduced activation, alongside an inability to induce replication-dependent immune signatures limiting the depth of the immune response. We also acknowledge the small sample size in these studies. Therefore, future work is needed further explore the direct cell mediated responses in the presence/absence of SGAs. Our *in vivo* studies clearly highlight an olanzapine mediated reduction of neutrophil recruitment and activation but olanzapine effects on bone marrow were beyond the scope of this study. Therefore, future work is required to assess whether olanzapine induced impairment of immune cell function if mediated through either direct drug-receptor interactions or through changes of immune cell production and maturation from hematopoietic stem cells within the bone marrow. In the pharmacoepidemiological analysis, whilst ICS prescribing was used as a proxy measure of prior lung disease, indication for prescription was seldom reported to the NPSUM. Furthermore, smoking status could not be considered as this is not routinely reported to the NPSUM.

Conclusion

This study provides mechanistic evidence for a direct impact of olanzapine therapy on the host immune response to infection with a particular effect observed on neutrophil motility and function. Whilst this immunomodulatory effect alone is insufficient to worsen the overall response to bacterial infection, when combined with lung damage consistent with chronic respiratory diseases and their associated therapies, the risk of pneumonia and associated disease severity is significantly elevated. The translational implications of this data require careful consideration. Clinically, it is important to recognise that patients receiving SGAs such as olanzapine, particularly those with chronic respiratory comorbidities may represent a patient subgroup that requires closer monitoring/interventions during respiratory infection. Future work involving observational clinical trials involving patients on long-term SGA beyond just olanzapine therapy will be important to establish whether and to what degree these preclinical results translate into clinical practice enabling a greater understanding of the associated risks and required monitoring needed with SGA therapy.

Declarations

Ethical approval and consent to participate - Ethical approval for healthy volunteer work was obtained from the University College London Research Ethics Committee (reference 19181/001). All animal experiments were performed in accordance with the Animals (Scientific) Procedures Act 1986 with 2012 amendment (PPL: P1P1060882), with local ethical approval from King's College London. Animal studies are reported in compliance with the ARRIVE guidelines.

The King's College London Biomedical and Health Sciences, Dentistry, Medicine and Natural and Mathematical Sciences Research Ethics Sub-Committee (BDM-RESC) re-confirmed in August 2026, that NPSUM does not require review as all subjects are deceased

Consent for publication – Not applicable

Availability of data and materials – The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests – The authors declare that they have no competing interests

Funding - This study was conducted as part of the PhD research of AS funded by the Centre for Pharmaceutical Medicine Research at King's College London and did not receive any additional funding.

Author Contributions

AS: Data curation (lead); formal analysis (equal); investigation (equal); methodology (supporting); writing-original draft (supporting).

SS: Data curation (supporting); investigation (supporting); methodology (supporting); writing-review and editing (supporting)

AK: Data curation (supporting); investigation (supporting); methodology (supporting); writing-review and editing (supporting).

BR: Data curation (supporting); investigation (supporting); methodology (supporting); writing-review and editing (supporting).

CP: Formal analysis (supporting); investigation (supporting); methodology (supporting); ; writing-review and editing (supporting).

DT: Formal analysis (supporting); investigation (supporting); methodology (supporting); ; writing-review and editing (supporting).

NA: Data curation, investigation, methodology; writing-review and editing (supporting).

TS: Data curation, investigation, methodology; writing-review and editing (supporting).

MS: conceptualisation (supporting); funding acquisition (supporting); resources (supporting); supervision (supporting); writing-review and editing (supporting); writing-review and editing (supporting).

CC: conceptualisation (equal); data curation (equal); formal analysis (supporting); funding acquisition (equal); methodology (supporting); project administration (equal); resources (equal); supervision (equal); writing-original draft (supporting); writing-review and editing (supporting).

RA: Conceptualisation (equal); data curation (equal); formal analysis (equal); funding acquisition (supporting); investigation (lead); methodology (equal); project administration (equal); resources (lead); supervision (equal); writing-original draft (lead); writing-review and editing (lead).

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Figures

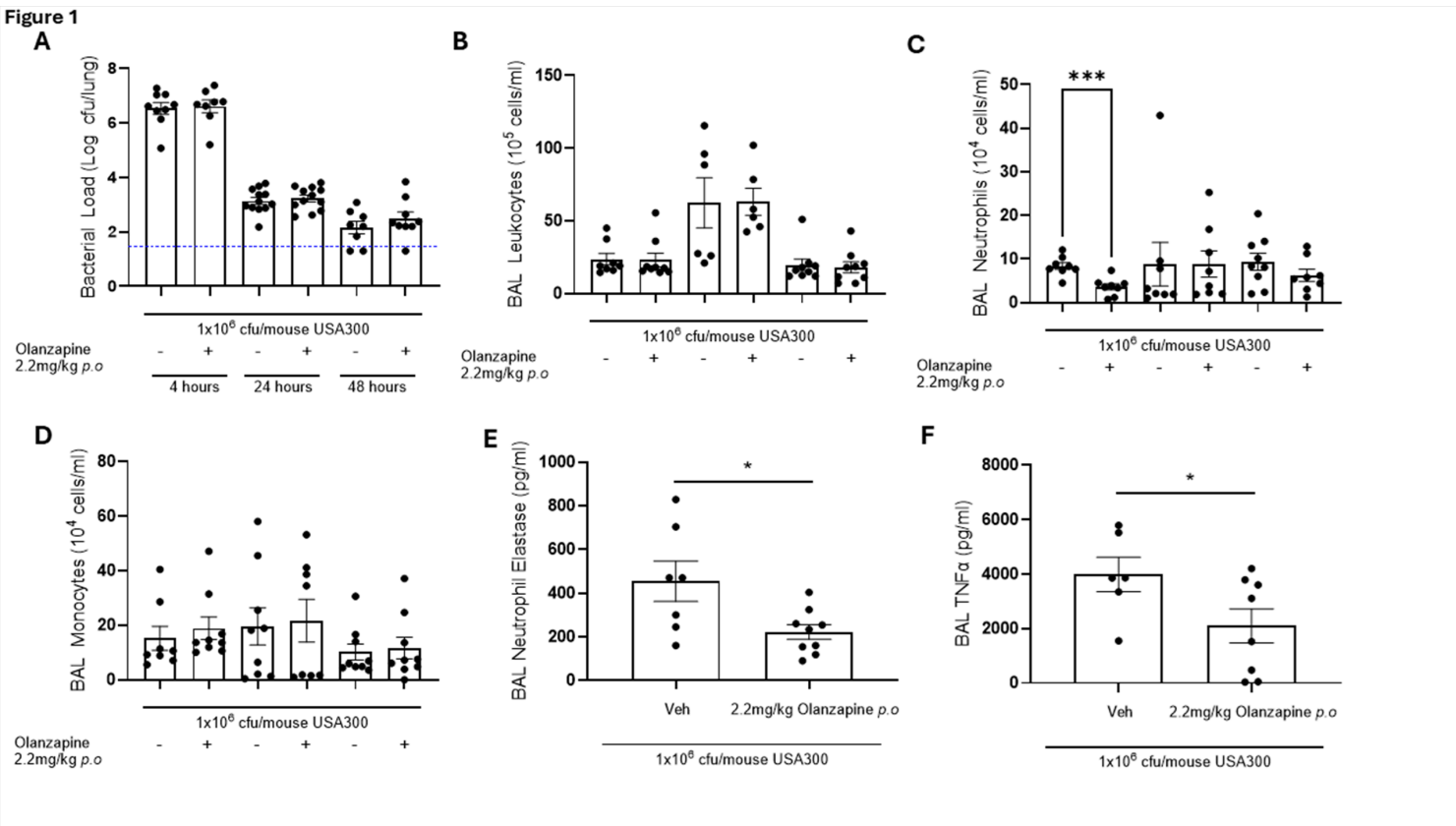


Figure 1

Olanzapine therapy impairs neutrophil recruitment to the lungs following pulmonary infection with USA300. C57Bl/6 mice were treated daily with 2mg/kg olanzapine or vehicle control for 16 days via oral gavage. On day 14, animals were inoculated with 1×10^6 cfu/mouse of the *S. aureus* strain USA300 via *i.t.* administration. Animals were culled at 4, 24 and 48 hours post infection. Lungs were aseptically removed for quantification of pulmonary bacterial load (A). Total leukocytes (B), neutrophils (C) and monocytes (D) were quantified in BAL fluid. At 24 hours post infection Neutrophil Elastase (E) and TNF α (F) levels were quantified in BAL fluid via ELISA. Data are represented as mean $\pm$ SEM with $n = 6-12$ across 2 independent studies. Data were analysed using a one-way ANOVA and a Sidaks multiple comparison post-test. * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

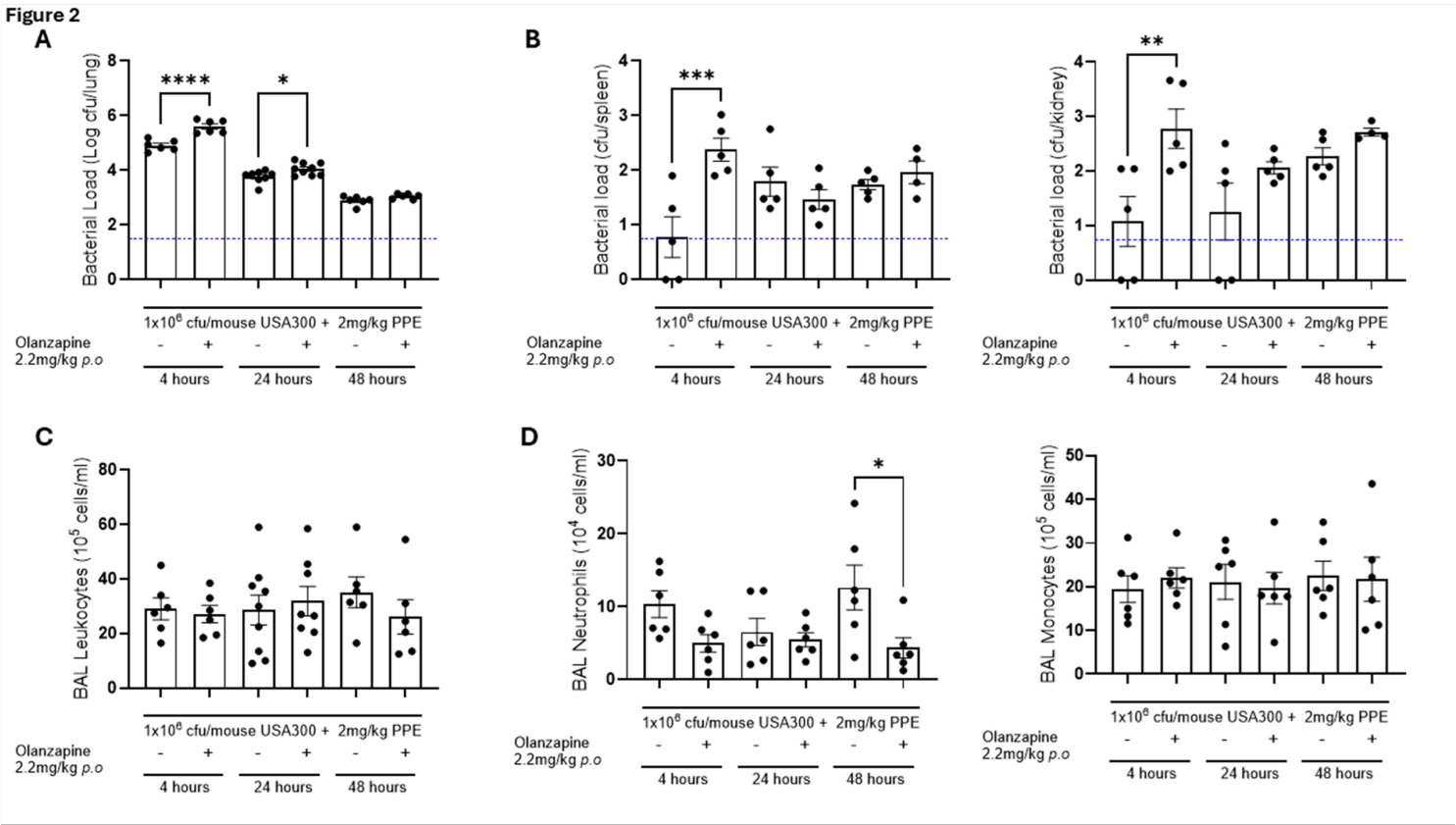


Figure 2

Olanzapine significantly worsens acute pulmonary infection with USA300 in a model with prior lung injury associated with chronic respiratory diseases. C57Bl/6 mice were treated daily with 2mg/kg olanzapine or vehicle control for 16 days via oral gavage. On days 4, 8 and 12, mice were challenged with 2mg/kg PPE via intranasal administration. On day 14, animals were inoculated with 1×10^6 cfu/mouse of the *S. aureus* strain USA300 via *i.t.* administration. Animals were culled at 4, 24 and 48 hours post infection. Lungs (A), Spleen (B) and Kidneys (C) were aseptically removed for quantification of pulmonary bacterial load and evidence of systemic bacterial dissemination. Total leukocytes (D), neutrophils (E) and monocytes (F) were quantified in BAL fluid. Data are represented as mean $\pm$ SEM with $n = 5-6$. Data were analysed using a one-way ANOVA and a Sidaks multiple comparison post-test. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ and **** = $P < 0.0001$.

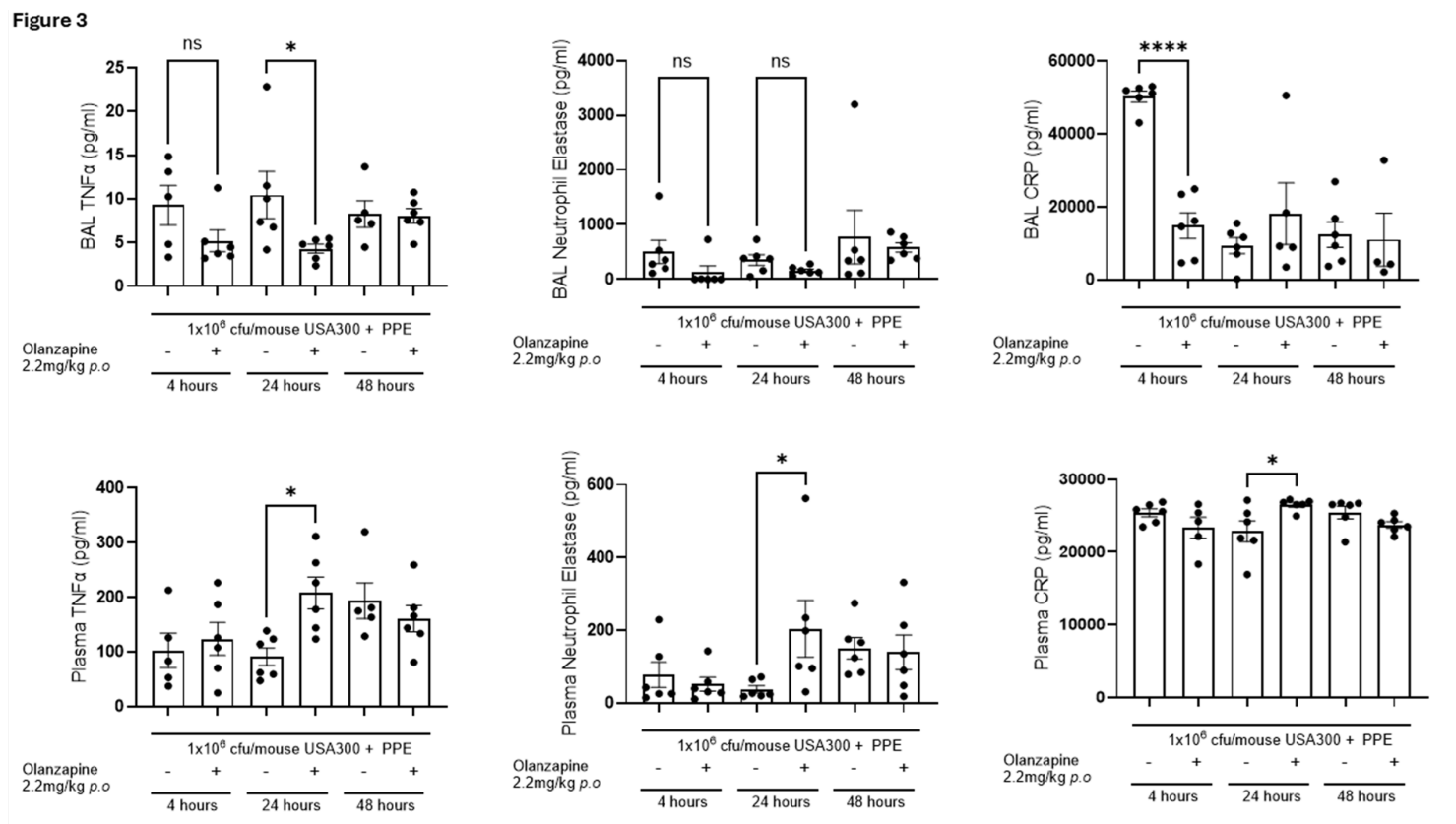


Figure 3

Olanzapine significantly alters pulmonary and systemic cytokine mobilisation following pulmonary infection with USA300 in a model with prior lung injury associated with chronic respiratory diseases. C57Bl/6 mice were treated daily with 2mg/kg olanzapine or vehicle control for 16 days via oral gavage. On days 4, 8 and 12, mice were challenged with 2mg/kg PPE via intranasal administration. On day 14, animals were inoculated with 1×10^6 cfu/mouse of the *S. aureus* strain USA300 via *i.t.* administration. The inflammatory cytokines TNFα, Neutrophil Elastase and CRP were measured both in BAL fluid (**A-C**) and in the systemic circulation (**D-F**) via ELISA. Data are represented as mean $\pm$ SEM with $n = 5-6$. Data were analysed using a one-way ANOVA and a Sidaks multiple comparison post-test. * = $P < 0.05$, and **** = $P < 0.0001$.

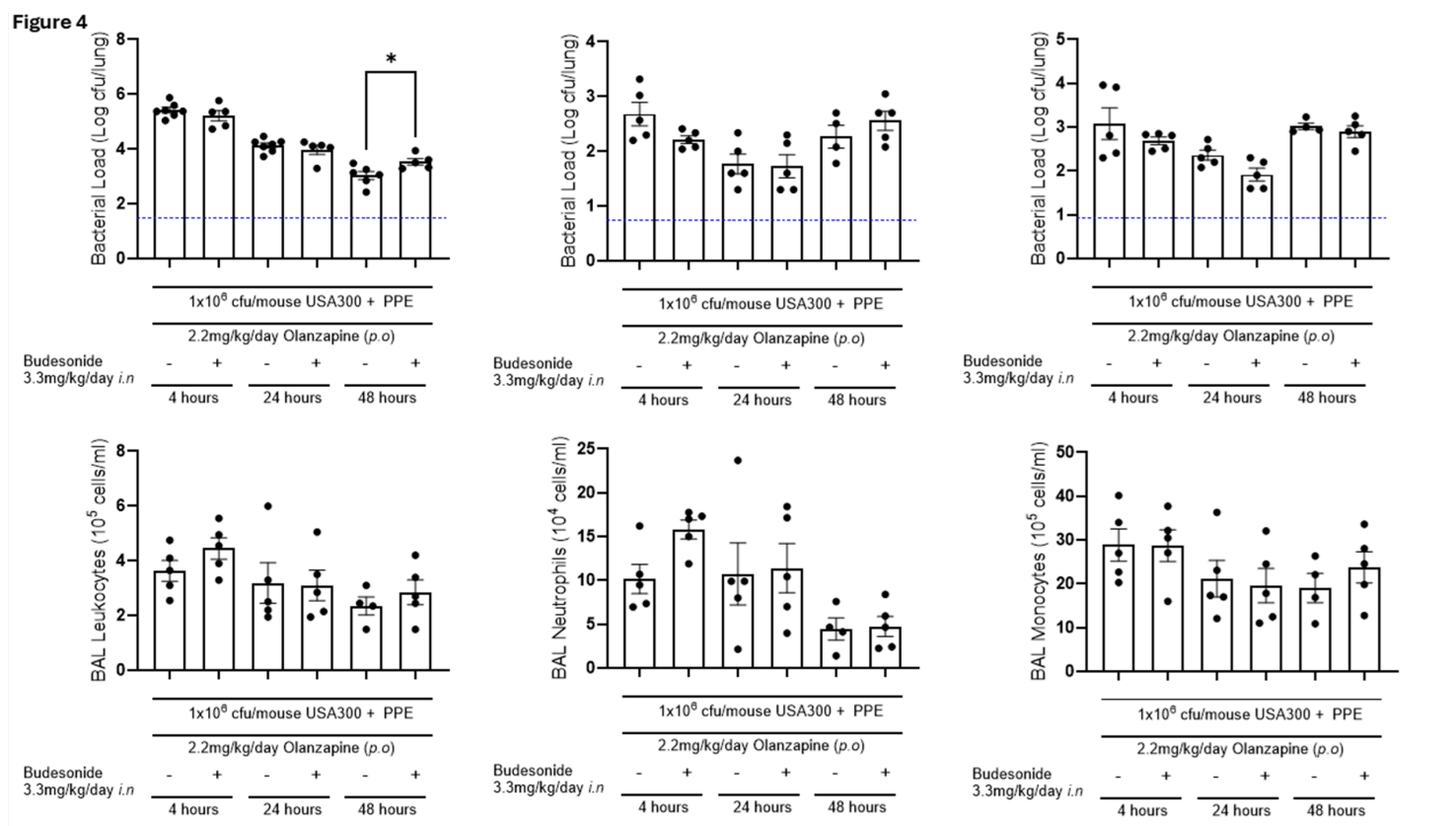


Figure 4

Combined olanzapine and budesonide treatment delays bacterial clearance following acute pulmonary infection with USA300 in a model with prior lung injury associated with chronic respiratory diseases.

C57Bl/6 mice were treated daily with 2mg/kg olanzapine, 3.3mg/kg Budesonide, vehicle control and combinations thereof for 16 days via oral gavage. On days 4, 8 and 12, mice were challenged with 2mg/kg PPE via intranasal administration. On day 14, animals were inoculated with 1x10⁶ cfu/mouse of the *S. aureus* strain USA300 via *i.t.* administration. Animals were culled at 4, 24 and 48 hours post infection. Lungs (A), Spleen (B) and Kidneys (C) were aseptically removed for quantification of pulmonary bacterial load and evidence of systemic bacterial dissemination. Total leukocytes (D), neutrophils (E) and monocytes (F) were quantified in BAL fluid. Data are represented as mean ± SEM with *n* = 5-6. Data were analysed using a one-way ANOVA and a Sidaks multiple comparison post-test. * = *P*<0.05, ** = *P*<0.01, *** = *P*<0.001 and **** = *P*<0.0001.

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