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# Sodium Pyruvate Ameliorates Influenza A Virus Infection In Vivo

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**Abstract:** Influenza A virus (IAV) causes seasonal epidemics annually and pandemics every few decades. Most antiviral treatments used for IAV are only effective if administered during the first 48 h of infection and antiviral resistance is possible. Therapies that can be initiated later during IAV infection and that are less likely to elicit resistance will significantly improve treatment options. Pyruvate, a key metabolite, and an end product of glycolysis, has been studied for many uses, including its anti-inflammatory capabilities. Sodium pyruvate was recently shown by us to decrease inflammasome activation during IAV infection. Here, we investigated sodium pyruvate's effects on IAV in vivo. We found that nebulizing mice with sodium pyruvate decreased morbidity and weight loss during infection. Additionally, treated mice consumed more chow during infection, indicating improved symptoms. There were notable improvements in pro-inflammatory cytokine production (IL-1β) and lower virus titers on day 7 post-infection in mice treated with sodium pyruvate compared to control animals. As pyruvate acts on the host immune response and metabolic pathways and not directly on the virus, our data demonstrate that sodium pyruvate is a promising treatment option that is safe, effective, and unlikely to elicit antiviral resistance.

**Keywords:** pyruvate; inflammation; influenza A virus; antiviral



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## 1. Introduction

Influenza A virus (IAV) causes seasonal epidemics and periodic pandemics with significant morbidity and mortality. In the 2019–2020 flu season, the United States Center for Disease Control and Prevention (CDC) estimated 38 million IAV infections and 22,000 deaths. The most prevalent virus of the 2019–2020 season was the 2009 pandemic IAV (H1N1). Notably, during this season, was a higher rate of infections among children aged 0–4 and adults aged 18–49 years than in other recent seasons [1]. During pandemics, the emergence of novel viruses can cause severe complications with increased morbidity and mortality [2]. Due to the novelty of pandemic viruses, vaccines must be redesigned. Anti-viral therapies exist to treat IAV [3]. However, viral resistance to these therapies is always possible. Therefore, treatments that alter the host response to IAV infection and are less likely to result in the evolution of resistance are desirable.

Studies have shown that IAV hijacks the cellular metabolism to increase viral replication [4,5]. Pyruvate (Pyr) ($C_3H_4O_3$) is a central metabolite and a key component in energy metabolism and cellular respiration. Pyr can enter directly into the mitochondria to produce ATP via the tricarboxylic acid cycle (TCA), which bypasses many of the metabolic regulatory pathways that control glycolysis [6,7]. Mitochondrial oxidative phosphorylation is the most efficient way to produce ATP for cells. However, Pyr can also be used to make amino acids or be reduced to lactate via fermentation or the Warburg effect [8,9]. The reduction of Pyr is used to replenish Nicotinamide adenine dinucleotide ($NAD^+$) and increase the uptake of necessary nutrients for rapidly dividing cells, such as immune and cancer cells [8–10]. The end goal is rapid proliferation, not energy efficiency, in most of these cases [10].

Pyr in its many forms (ethyl Pyr, pyruvic acid, pyruvate anion, sodium pyruvate, etc.) has been found to have many antioxidant-like benefits in several body systems. The molecule seems to be well tolerated in the body with little to no toxicity [11]. Ringer's ethyl pyruvate has been used primarily for its increased stability in solution, however, hypertonic sodium pyruvate has been found to more effectively protect against inflammation and stress during injury events [12]. Additionally, Pyr has been found to have a plethora of beneficial effects on the cardiac system [13–15]. Moreover, increasing extracellular Pyr in the brain has been found to decrease neuronal death during traumatic brain injury events [16,17] and be protective to neurotoxic compounds [18]. When administered to mice of various ages, Pyr increased glycogen stores and brain energy metabolites, which could help with diseases such as Alzheimer's [19]. Additionally, Pyr decreases epithelial permeability, inflammation, and bacterial translocation during intestinal ischemic reperfusion (I/R) events [20,21]. Decreased damage during I/R events has also been shown to be beneficial during organ transplantation because of decreased organ damage [22] and damage caused by rejection following transplantation [23]. In addition to having benefits in body systems, sodium pyruvate (NaPyr) has been shown to help with organ storage for transplant surgeries by decreasing cell death, overall improving metabolism during cold storage [24,25], and increasing graft metabolism [26]. Red blood cell (RBC) storage has been known to generate reactive oxygen species (ROS), but the addition of NaPyr to the storage media decreases ROS and increases antioxidant enzymatic, superoxide dismutase (SOD), activity which leads to an overall higher RBC recovery rate [27]. RBCs stored with varying levels of Pyr maintain 2,3-diphosphoglycerate (2,3-DPG) production for longer periods of storage [28]. Bone and tissue inflammation models have shown that Pyr treatment leads to less destructive disease via anti-inflammatory properties [29,30]. In relation to infectious disease, sodium pyruvate (NaPyr) ($C_3H_4NaO_3$) can improve herpes simplex 2 virus infection in vivo, and our lab recently reported that NaPyr can regulate inflammation during IAV infection in vitro [31,32].

In our previous study, we observed in mouse bone marrow-derived macrophages (BMDM), that NaPyr has anti-inflammatory capabilities through altered metabolism [31]. The addition of NaPyr to BMDM decreased mitochondrial damage in response to IAV infection. These findings led us to further investigate NaPyr's potential anti-viral and anti-inflammatory capabilities in a mouse model of IAV infection. Here, we show that nebulizing NaPyr in vivo in wildtype (WT) C57BL/6J mice leads to decreased weight loss and increased chow intake over the course of IAV infection. Seven days post infection (p.i.), animals treated with NaPyr displayed decreased pro-inflammatory cytokines (IL-1β) in the lungs and decreased virus replication.

## 2. Materials and Methods

### *2.1. Animal Welfare*

WT C57BL/6J mice were bred and raised in the Temple Hall Vivarium at Missouri State University. Mice were euthanized via $CO_2$ asphyxiation and cervical dislocation or cardiac puncture at humane end points or tissue collection. All breeding and experiment protocols were performed in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines (protocols 19.005 and 19.019), the AVMA Guidelines on Euthanasia, NIH regulations (Guide for the Care and Use of Laboratory Animals), and the U.S. Animal Welfare Act of 1966.

### *2.2. Virus Production*

The strain of IAV used in all experiments was influenza A/PR/8/34 H1N1 (PR8). PR8 stocks were generated by infecting pathogen-free hen's eggs with 1000 PFU of PR8. Following a 3-day incubation, the allantoic fluid was harvested, centrifuged to remove debris, and stored at −80 °C for later use.

### *2.3. In Vivo Infection and NaPyr Treatments*

Mice were anesthetized on Day 0 via intraperitoneal injection of 80 mg/kg of Ketamine and 8 mg/kg of Xylazine. Mice were then infected intranasally with approximately 250 PFU of influenza A/PR/8/34 H1N1, diluted in 30 µL of phosphate buffered saline (PBS). Mice used for subcutaneous (Sub-Q) injections of NaPyr were injected with 110 mg/kg of body weight daily, divided into two doses, morning and evening. Mice that were treated with nebulized NaPyr were treated with Emphycorp's clinical grade N115 (20 mM of NaPyr), or with 10 mM of NaPyr (Fisher Bioreagents, Pittsburgh, PA, USA, BP356-100) diluted in PBS, or treated with PBS alone as control. Mice were treated three times a day for 20 min per treatment. All mice were monitored for food/water availability and weighed daily for weight loss and/or becoming moribund. Mice were euthanized if the experiment was on day 14, or the day of sacrifice for tissue samples. Food intake was also monitored by weighing the food daily and averaging the change in food weight by the number of animals per cage.

### *2.4. Tissue Collection and Processing*

Mice sacrificed for tissue samples on day 3 and day 7 p.i. were euthanized via $CO_2$ asphyxiation and cardiac puncture as an adjunct. Lungs were taken from sacrificed mice for processing. Lungs were weighed and homogenized through a 70 µm cell strainer (Fisherbrand, 22363548) with a final volume of 4 mL of RPMI 1640 without serum or NaPyr (Hyclone, Logan, UT, USA, SH30027.01) per tissue sample. Samples were then centrifuged and aliquoted for future use.

### *2.5. Flow Cytometry for Innate and Adaptive Immune Cells*

Lung homogenates were centrifuged at 400× *g* for 7 min to achieve cell pellet. After the removal of the supernatant for other assays, red blood cells were lysed with ACK lysis buffer. Dead cells and debris were then removed by centrifugation in 37.5% Percoll (GE Healthcare, Chicago, IL, USA, 17-0891-02) at 2000× *g* for 20 min. Cells were then stained with fluorescent antibodies (Table 1). Samples were run on the Accuri C6 Flow Cytometer.

**Table 1.** Fluorescent antibodies used for FACS staining in preparation for flow cytometry.

| Fluorophore | Monocyte Stain | Cat# | Lymphocyte Stain | Cat# |
|---|---|---|---|---|
| FITC | CD11c | 35-0114-U100 | CD4 | 35-0042-U100 |
| PE | Gr1 | 50-5931-U100 | CD8 | 100707 |
| PerCP 5.5 | CD3ε | 65-0031-U100 | CD3ε | 65-0031-U100 |
| APC | CD11b | 20-0112-U100 | CD19 | 115511 |

Antibodies were purchased from Tonbo (San Diego, CA, USA) or Biolegend (San Diego, CA, USA).

### *2.6. Viral Plaque Assay*

The IAV plaque assay was performed using Madin-Darby Canine Kidney (MDCK) cells seeded at $2 \times 10^5$ cells/well in 12-well plates in DMEM + 5% FBS + 1% Pen/Strep. 10-fold dilutions of the virus were prepared in RPMI 1640. MDCK cells were washed with PBS twice and 100 µL of each virus dilution was added to wells in 12-well plates and incubated at 37 °C and 5% $CO_2$ for one hour. Semisolid overlay was prepared as previously described [33]. TPCK trypsin was added to a final concentration of 1.0 µg/mL. After a full hour of incubation, the infection medium was removed from 12-well plates, 2 mL of the warm overlay with TPCK trypsin was added to each well and allowed to solidify. Plates were turned upside down and incubated for 3 days at 37 °C and 5% $CO_2$. After incubation, the overlay was removed, and plaques counted after staining with 1% crystal violet in formalin.

### *2.7. Enzyme-Linked Immunosorbent Assay (ELISA)*

Supernatant from homogenized lung tissue samples were analyzed for IL-1β and IL-6. ELISA kits were purchased from Ebioscience (88-7013-88, 88-7064-88) and assays performed

according to the manufacturer's recommendations. Plates were read at 450 nm on a microplate reader (BioTek ELx808, Winooski, VT, USA). Cytokine levels were normalized to lung weight/mL.

### *2.8. Statistical Analysis*

Statistical analysis was performed using GraphPad PRISM9. For in vivo weight loss and chow consumption during experiments, a two-way ANOVA was performed. For viral titer, cytokine and cell populations analysis, a Student's *t*-test was performed. A $p$-value < 0.05 was considered statistically significant.

## 3. Results

### *3.1. NaPyr Is Not Toxic In Vivo*

N115 is a clinical grade nasal spray containing 20 mM of NaPyr that has undergone safety and phase I, phase II and phase III clinical trials. The FDA is currently reviewing the administration of N115 for use in chronic obstructive pulmonary disease (COPD) patients with idiopathic pulmonary fibrosis, or idiopathic pulmonary fibrosis patients alone (EmphyCorp (Flemington, NJ, USA), Cellular Sciences Inc. (Newburyport, MA, USA) FDA submissions). Patient surveys indicated that use of N115 may decrease the incidence, symptoms, and duration of respiratory infections too. Millions of patients have been treated with N115 nasal spray in over 200 hospitals globally, which includes pregnant women, patients with allergic rhinitis, COPD patients, patients with sinusitis, and patients with pulmonary fibrosis, with no adverse events reported. The use of the nasal spray in these patients demonstrates its safety and efficacy and the ability of NaPyr to reduce nasal congestion and inflammation. In a phase III placebo controlled clinical trial with idiopathic pulmonary fibrosis patients, the N115 nasal spray demonstrated a statistically and clinically significant increase in nasal nitric oxide, FEV-1, SaO2, FVC, FEV-1/FVC ratios (52% to 86%). N115 reduced hypoxemia, and it also reduced lung inflammation, inflammatory cytokines, and coughing. Other studies confirm the safety of supplementation with NaPyr [11,17,34].

Based on these promising data, we sought to examine the effectiveness of NaPyr for the treatment of IAV infection. We conducted preliminary toxicity experiments using nebulized NaPyr at 10 mM and 1 M concentrations made in house using Fisher Bioreagents NaPyr (BP356-100) diluted in PBS, or PBS for control. We found no noticeable weight loss in mice treated with 10 mM of NaPyr. 1 M of NaPyr treatment did show some slight decline in weight in mice, but this was likely due to the cloud produced by nebulizing 1 M of NaPyr, which was thick like chalk dust and difficult to breathe (Figure 1). Overall, NaPyr was not found to be toxic at the concentrations used for the treatment of IAV infected mice.

### *3.2. Nebulized NaPyr Improves Weight Loss in IAV Infected Mice*

Our previous research with NaPyr in vitro established its immunomodulatory properties [31]. We, therefore, examined its effects in mice infected with IAV. WT C57BL/6J mice were infected with 250PFU of influenza A/PR/8/34 H1N1 virus and injected sub-cutaneously (Sub-Q) with 55 mg/kg of NaPyr twice a day for 14 days and compared to PBS injection controls. Although the injection of NaPyr resulted in increased food intake early and late during infection, it did not significantly improve weight loss in IAV infected mice (Figure 2A,B). Thus, we began looking for an alternative, more direct administration method during infection. Aerosols are used frequently for the treatment of lower respiratory infections, more specifically, viral pneumonia [35]. Hence, we hypothesized that a nebulization model would be a more direct route to the site of infection. Intriguingly, treating mice three times a day with nebulized 10 mM of NaPyr for approximately 15-min intervals, resulted in some improvement in weight loss and increase in food intake (Figure 2C,D). (Data in Figure 2C,D are combined from two independent experiments with $n$ = 4–5 mice per treatment group per experiment. Two-way ANOVA $p$ = 0.0399, 0.0043, 0.0116, and 0.0363 for days 5–8 in Figure 2C, Two-way ANOVA $p$ = 0.0199 and 0.0093 for days 2–3 in Figure 2D).

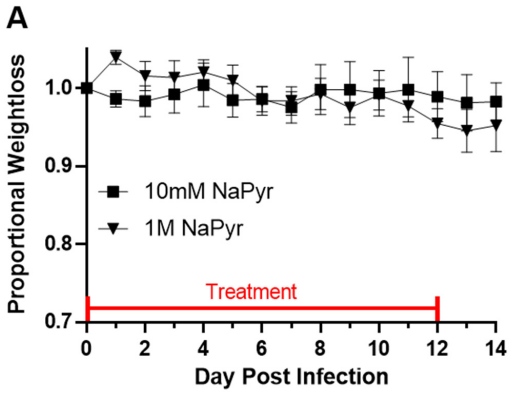


**Figure 1.** Sodium pyruvate (NaPyr) shows no toxicity in mice. WT C57BL/6J mice were nebulized 3 times daily for 15 min per treatment with 10 mM and 1 M concentrations of NaPyr diluted in phosphate buffered saline (PBS) for 14 days to determine toxicity and weight differences between treatment groups. Data are representative of one experiment with $n$ = 5 mice per treatment group.

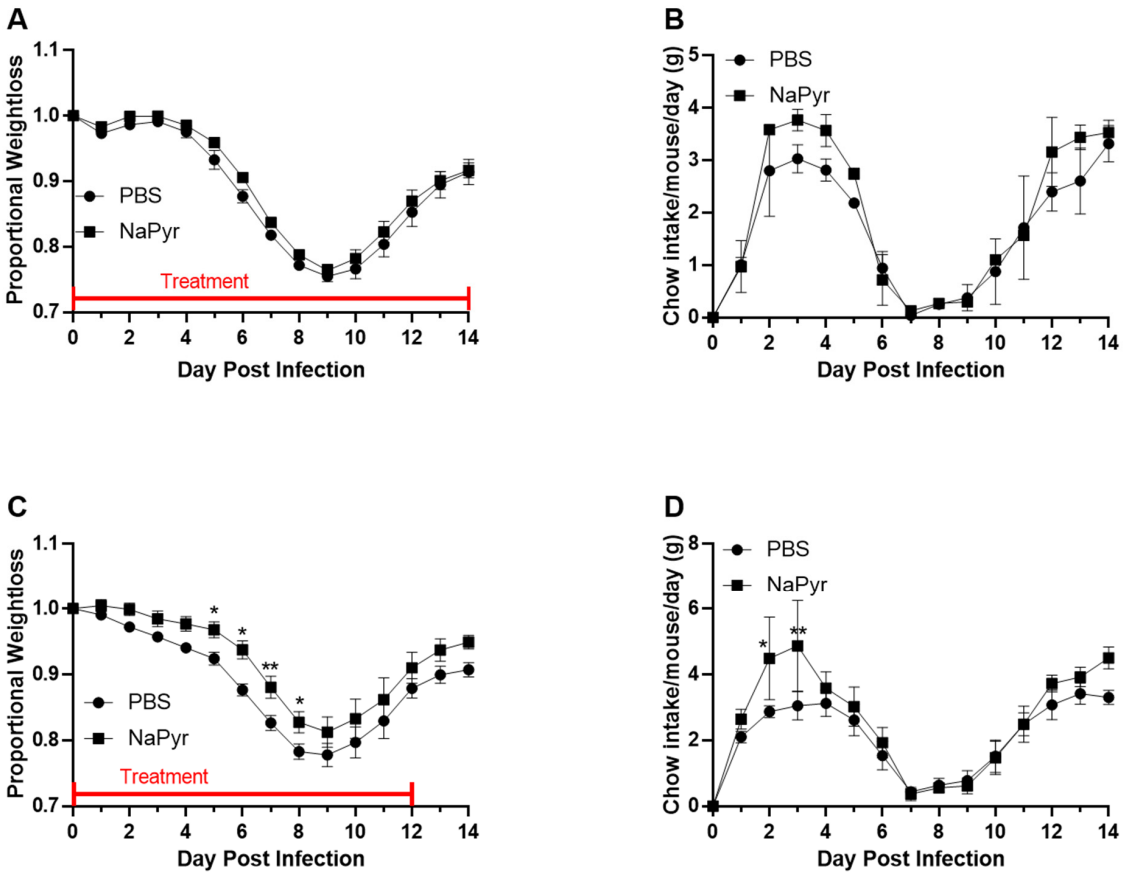


**Figure 2.** Effects of injection or nebulization of NaPyr on influenza A virus (IAV) infection. WT C57BL/6J mice were infected intranasally with 250 PFU of influenza A/PR/8/34 H1N1. Mice were treated as indicated and monitored daily for 14 days to determine survival and weight differences between treatment groups. (**A**) Weight loss was examined in mice injected Sub-Q with 55 mg/kg NaPyr twice a day for 14-days compared to PBS injected mice. (**B**) Average chow intake over the 14-day IAV infection of both Sub-Q NaPyr treated and PBS treated mice. (**C**) Mice were treated 3 times a day with either nebulized 10 mM NaPyr or nebulized PBS as control. Weight loss differences viewed over the 14-day IAV infection of both NaPyr treated and PBS treated mice. (**D**) Average chow intake over the 14-day IAV infection of both nebulized 10 mM NaPyr and PBS treated mice. Data are representative of 2–3 individual experiments with $n$ = 4–5 mice per treatment group per independent experiment. Statistical significance was determined using a Two-way ANOVA with Fisher LSD post-hoc for multiple comparisons. * $p < 0.05$, ** $p < 0.01$.

### *3.3. N115 Decreases Weight Loss and Increases Chow Intake during IAV Infection*

As stated above, EmphyCorp manufactures a stable 20 mM NaPyr nasal spray (N115). In phase I/II/III clinical trials, N115 has demonstrated promising results in decreasing lung inflammation in COPD and idiopathic pulmonary fibrosis patients. Using N115, we examined potential toxicity, but observed no difference in weight loss between uninfected mice treated with N115 compared to PBS controls (Figure 3A). Next, we examined weight loss in WT C57BL/6J mice infected with 250PFU of influenza A/PR/8/34 H1N1 virus and treated three times a day for 20-min intervals. Our results indicate that nebulizing mice with N115 over the course of 12 days of IAV infection decreased weight loss and increased chow intake, compared to the PBS controls. We found days 7–14 to be statistically significant between the control and N115 treated groups (Figure 3B). Chow intake in the N115 treated mice was significantly higher on days 9–10 too (Figure 3C). (Weight loss and chow intake data are combined from three independent experiments with $n$ = 4–6 mice per treatment group per independent experiment. Two-way ANOVA $p$ = 0.0127, 0.0012, 0.0002, <0.0001, 0.0005, 0.0046, 0.0233, 0.0311 for days 7–14, respectively, in Figure 3B. Two-way ANOVA $p$ = 0.0492 and 0.0335 for days 9–10 in Figure 3C).

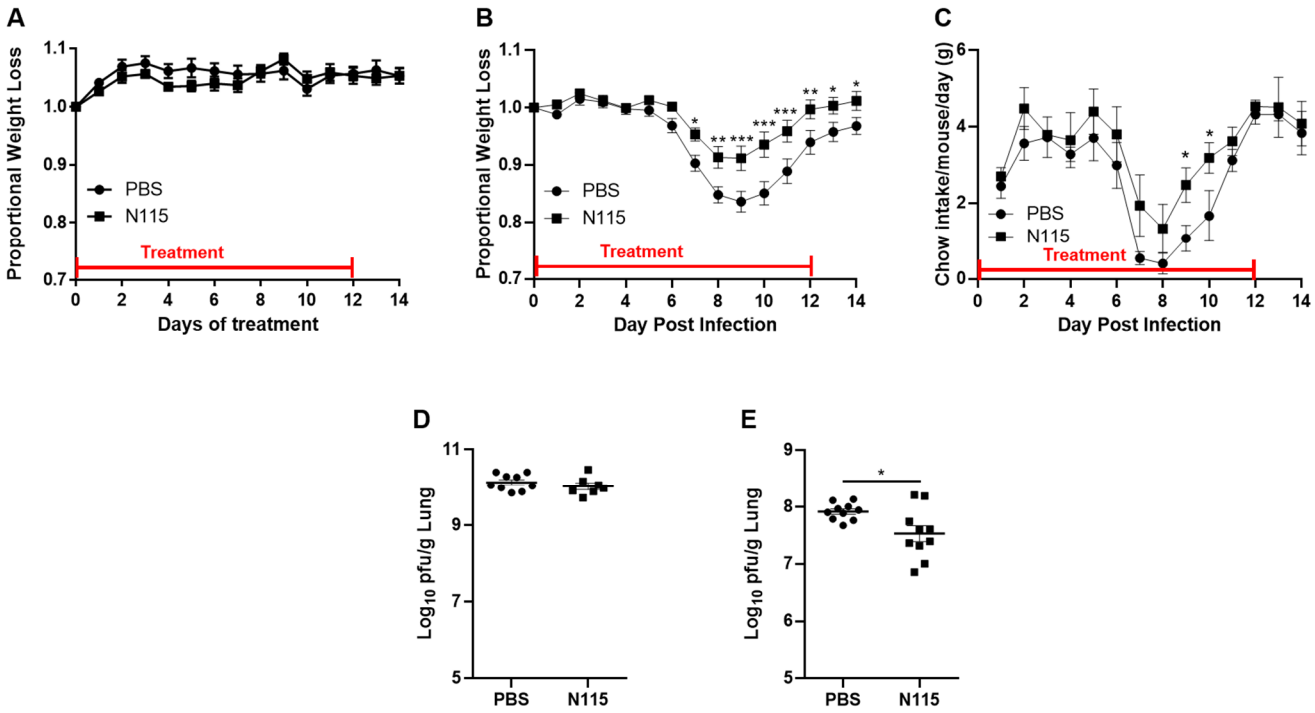


**Figure 3.** Nebulized N115 improves weight loss and virus titer in mice infected with IAV. WT C57BL/6J mice were treated with either nebulized 20 mM NaPyr (N115) or nebulized PBS as control 3 times a day for 20 min/treatment to test for toxicity (**A**). WT C57BL/6J mice were infected intranasally with 250 PFU of influenza A/PR/8/34 H1N1. Mice were treated with either nebulized 20 mM NaPyr (N115) or nebulized PBS as control 3 times a day for 20 min/treatment. (**B**) Mice were monitored daily for 14 days to determine weight differences between treatment groups. (**C**) Average chow intake over the 14-day IAV infection of both N115 treated and PBS treated mice. (**D**,**E**) Viral titer was assessed by plaque assay on day 3 (**D**) and day 7 (**E**) p.i. from lung homogenates. Weight loss and chow intake data are representative of 3 independent experiments with $n$ = 4–6 mice per treatment group per independent experiment. Viral titer data are representative of 2 individual experiments with 3–5 mice per treatment group per individual experiment. Statistical significance was determined using a Two-way ANOVA with Fisher LSD post-hoc for multiple comparisons, and Student's $t$-test for single comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

As N115 improved weight loss, we next examined the cause for improved weight loss. We investigated viral titers by plaque assay on day 3, before weight loss, and day 7 p.i., just as the N115 treated mice started to show improvement in weight loss. We found

that there was significantly less virus in the lungs of N115 treated mice compared to PBS treated controls on Day 7 (Figure 3D,E). (Viral titer data are combined from two individual experiments with 3–5 mice per treatment group per experiment. Statistical significance was determined using a Student's *t*-test, $p = 0.0172$ for Figure 3E).

As previously reported in vitro [31], we also observed significantly less IL-1β levels in the lungs of N115 treated mice (Figure 4A,B). Despite lower IL-1β levels, most leukocyte numbers were similar in the lungs of N115 and PBS treated mice, except for inflammatory monocyte numbers, which were elevated in the N115 mice (Figure 4C,D). Overall, N115 appears to improve disease during IAV infection by decreasing virus titers and lowering inflammatory cytokine levels. (Data in Figure 4A–D are combined from two independent experiments with $n = 3$–5 mice per experiment. Statistical significance was determined using a Student's *t*-test. Figure 4A $p = 0.00672$ for IL-1β; Figure 4B $p = 0.0351$ for IL-1β; Figure 4C $p = 0.0442$ for inflammatory monocytes).

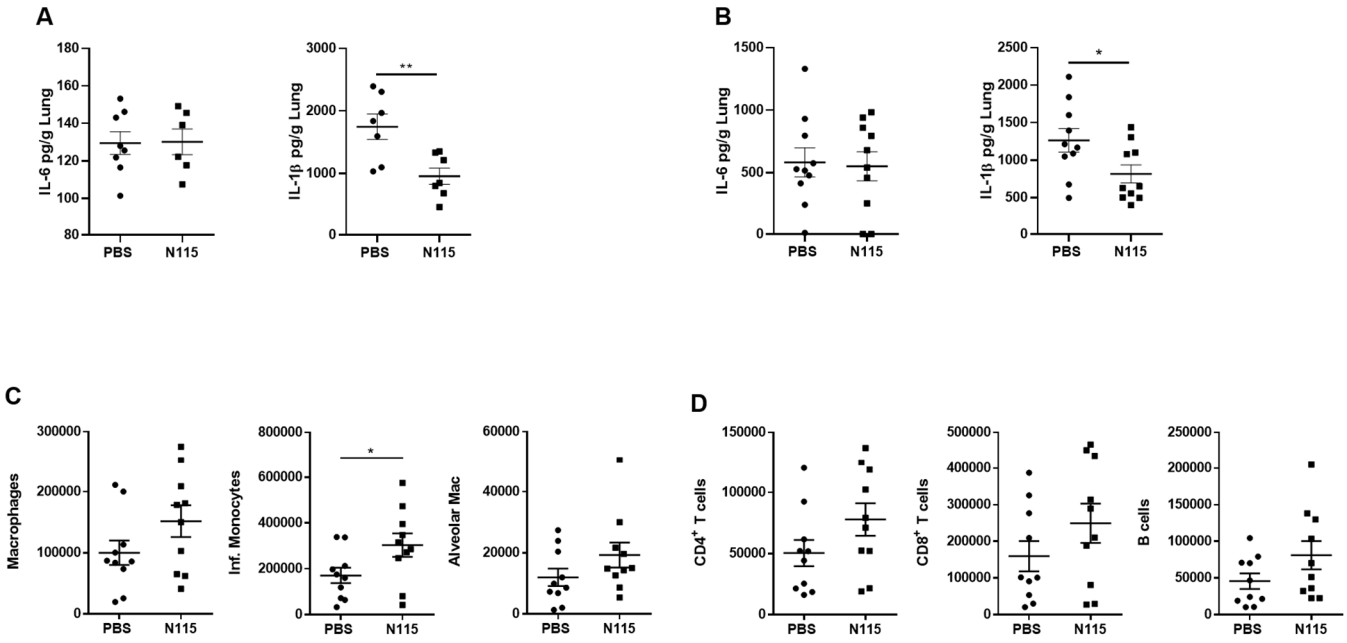


**Figure 4.** N115 treatment modulates inflammatory responses during IAV infection. WT C57BL/6J mice were anesthetized and infected with 250 PFU of influenza A/PR/8/34 H1N1. Mice were treated 3 times a daily for 20 min/treatment with either nebulized 20 mM NaPyr (N115) or nebulized PBS as control. Mice were euthanized on day 3 (**A**) or day 7 p.i. (**B**–**D**) for tissue collection. Lung samples were then homogenized and examined via ELISA for cytokine production (**A**,**B**) or cellular infiltration into the lungs by flow cytometry (**C**,**D**). Data are combined from 2 independent experiments with $n = 3$–5 mice per experiment. Statistical significance was determined using a Student's *t*-test for single comparisons. * $p < 0.05$, ** $p < 0.01$.

## 4. Discussion

Due to the evolution of antiviral or antibiotic resistance, the development of therapies that target host pathways to disrupt pathogen replication or disease is an avenue worthy of exploration. Some cellular metabolites can alter inflammation or pathogen replication, but our data suggest that the route of administration is important. Our data indicate that sub-Q injection of NaPyr does not influence IAV induced weight loss. However, nebulizing NaPyr does have a significant impact on weight loss, virus titer, and cytokine production during IAV infection in vivo in mice. Since Pyr can be rapidly absorbed by virtually any cell, injected NaPyr is likely taken up by other cells before reaching the target cells in the IAV infected lung [36,37]. Most IAV antiviral treatments target specific proteins within the virus. These proteins are prone to mutations and resistance to such drugs. Certain strains of IAV are known to be resistant to the M2 and neuraminidase inhibitors [38]. Since NaPyr affects cellular metabolism and inflammation instead of directly targeting virus replication,

there is a much lower chance that the virus will develop resistance to NaPyr treatment. Influenza and COVID-19 are known to cause mortality and morbidity in the elderly and immunocompromised. However, it is often forgotten that both diseases afflict children, usually with mild symptoms. In rare cases, there is mortality caused by complications during IAV infection. Seasonally, influenza causes 7000–26,000 hospitalizations in children under five years old [39]. COVID-19 this year has resulted in 3240 hospitalizations in school-aged children [40]. To date, 51 children, aged less than 18, have died in the United States from complications with COVID-19 [40]. Comparatively, the CDC has reported a range of 37–188 deaths annually in children under five years old from complications caused by influenza infections [39]. Our data in this manuscript clearly demonstrate that N115 improves influenza disease. Furthermore, we have preliminary data that suggest it may work similarly during other respiratory virus infections, including COVID19/SARS-CoV-2. Proactive treatment with NaPyr is not toxic and could be of benefit to children that are afflicted by many respiratory viruses.

As previously noted in vitro, the addition of NaPyr reduces mitochondrial ROS, in turn reducing mitochondrial damage during the course of IAV infection [31]. This reduction could indicate decreased Nod-like receptor containing pyrin 3 (NLRP3) inflammasome activation, which would further explain why IL-1β is lower in the mice treated with N115. Further studies would be necessary to ensure that NaPyr is modulating the NLRP3 inflammasome in vivo. However, in vitro we demonstrated that the addition of NaPyr to the infection media of BMDMs led to lowered caspase-1 activation and decreased pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) [31]. Presumably, this would be a similar mechanism in vivo to be explored with mice treated with N115. Future directions would include identifying and investigating if nebulized NaPyr influences lasting immunity following IAV infection, if NaPyr alters or diminishes lung damage caused by IAV infection, protects against severe disease cases, as well as ensuring that NaPyr is acting as an immunomodulator as we demonstrated in vitro.

It is also not clear how NaPyr affects virus replication. We reported that NaPyr does not affect virus replication in macrophages in vitro [31], and we have examined IAV replication in MDCK cells in vitro and found no effect of NaPyr either (data not shown). One possible explanation is that NaPyr alters immune cell function, such as enhanced $T_h1$ responses. This would agree with the timing observed in our experiments where virus titers were only lower on day 7 and weight loss was improved after day 7 too. Alternatively, NaPyr may elicit a response from respiratory epithelial cells that is antiviral, such as nitric oxide (NO) production or increased interferon responses. Duplicating the viral replication in both respiratory epithelial cell lines and other innate immune cells is needed to further address these questions. Another potential avenue would be to explore carbon tracing to determine where NaPyr is being shuttled during the IAV infection. More studies would have to be carried out to ensure NaPyr, or N115's, ability to ameliorate IAV infection in other model organisms such as ferrets. Notably, N115 is currently in human clinical trials for a multitude of inflammatory lung diseases including chronic obstructive pulmonary disorder and COVID-19. These clinical trials may also shed light on the mechanisms involved during IAV infection.

In conclusion, we show that nebulizing mice with sodium pyruvate decreased morbidity and weight loss during infection. Additionally, treated mice consumed more chow during infection, indicating improved disease symptoms. There were notable improvements in pro-inflammatory cytokine production (IL-1β) and lower virus titers on days 7 post infection (p.i.) in mice treated with NaPyr compared to control animals. As NaPyr appears to act on the host immune response and metabolic pathways and not directly on the virus, sodium pyruvate is a promising treatment option that is safe, effective, and unlikely to elicit antiviral resistance.

**Author Contributions:** J.M.R. and C.R.L. performed the experiments, analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** All mouse breeding and experiment protocols were performed in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines (protocols 19.005 and 19.019), the AVMA Guidelines on Euthanasia, NIH regulations (Guide for the Care and Use of Laboratory Animals), and the U.S. Animal Welfare Act of 1966.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data are included in the figures of this study.

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