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Temporal Development and Resolution of Lung Oedema in C57BL/6 Mice Following 0.9% Saline Bolus

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Abstract

Introduction: Administration of intravenous fluid in hospital and pre-hospital care is considered essential, particularly for stabilizing patients with hemodynamic instability, and providing alternative methods to oral fluid administration. Considered an isotonic solution due to its similarity to plasma, saline at 0.9% concentration is the most administered fluid in hospital. However, studies have suggested that there is minimal benefit and potential detriment to patients in routine fluid administration. This study aimed to elucidate the temporal course of fluid induced organ edema in a murine model following rapid intravenous fluid administration.

Methods: Male C57BL/6 mice were anaesthetized before cannulation of the femoral vein and 60 ml/kg body weight of 0.9% saline administered over 20 mins. Mice were monitored under anesthesia for a further 10, 40, or 70 mins post fluid administration. Lung tissue was resected and outcome measures including pulmonary edema, inflammatory cell infiltrates, and alveolar wall thickness were assessed at each time. Other tissues including brain, liver, kidney, and muscle were resected to assess edema development.

Results: Pulmonary edema was evident in mice administered fluids at 30 mins and 1 h, with resolution occurring at 1.5 h. Cellular infiltrate and alveolar wall thickness increased in mice by 1 h and was maintained through to 1.5 h. A reduction in brain wet to dry weight ratio was evident at 1 h following fluid, suggestive of dehydration, which was resolved by 1.5 h. No evidence of fluid associated changes was observed in liver, kidney, or muscle tissue at any time point.

Conclusion: These results indicate tissue specific effects of 0.9% intravenous fluid which, in healthy animals, resolve within 90 min following bolus administration.

Keywords: Intravenous fluids; lung injury; oedema; 0.9% saline

Introduction

Administration of intravenous fluid in hospital and pre-hospital care is considered essential, particularly for stabilizing patients with hemodynamic instability, and providing alternative methods to oral fluid administration. Considered an isotonic solution due to its similarity to plasma, saline at 0.9% concentration is the most commonly administered fluid in hospitals [1], being utilized to maintain plasma sodium (Na^+) concentration, tonicity, and provide fluid resuscitation and replacement to patients [2,3]. However, studies have suggested that there is minimal benefit and potential detriment to patients in routine fluid administration [4,5].

The FEAST study showed that fluid administration increased the relative risk of mortality after fluid bolus in African children with severe sepsis by 45%, predominantly within 24 hours, when compared to usual care [6]. The Simplified Severe Sepsis Protocol 2 study demonstrated increased in-hospital and 28-day mortality in patients receiving large fluid boluses compared to smaller volumes [7]. The mechanisms attributed to negative outcomes from these studies have largely been speculative; however, secondary analysis of FEAST indicated that delayed cardiac collapse in resuscitation patients was responsible for increased mortality [8].

The regulation of fluid movement across the epithelial barrier of the alveoli is strict. In addition to restricting fluid movement into the lungs, the epithelial barrier is actively involved in removing fluid; a process termed alveolar fluid clearance [9]. This mechanism is important in patient outcome with functional alveolar fluid clearance being attributed to decreased rates of mortality in patients with edematous acute lung injury [10]. Rapidly administering 0.9% saline leads to the development of interstitial pulmonary edema [11,12], without necessitating the increase in Left Ventricular End Diastolic Pressure (LVEDP) typically associated with hydrostatic injury [5]. We have previously demonstrated that rapid infusion of a saline bolus increases alveolocapillary barrier permeability, leading to subsequent increase in pulmonary edema in both a rodent model and in healthy human volunteers [5]. However, the time course of this fluid induced pulmonary edema has not been examined directly.

The purpose of this study was to elucidate the temporal development and resolution of fluid induced pulmonary edema in a murine model following administration of 0.9% saline bolus. To achieve this, C57BL/6 mice were exposed to rapid infusion of saline

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bolus and respiratory outcomes evaluated at time points up to 90 min. Additionally, edema was assessed in several other tissues following saline bolus administration to examine whether fluid bolus induced edema is lung specific.

Materials and Methods

Ethics Approval

The study protocol was approved by the Flinders University Animal Welfare Committee (Approval number: 871/14). Principles of laboratory animal care were followed (NIH publication No. 86-23, revised 1985) which are in accordance to the National Health and Medical Research Council (NHMRC) Australian Code for the Care and Use of Animals for Scientific Purposes, 2013 and in line with the State Government of South Australia Welfare Act, 1985.

Animal Characteristics

Male C57BL/6 mice were purchased from the Flinders University School of Medicine Animal Facility and maintained in pathogen-free conditions. All mice were 10-20 weeks old, weighed between 25-32 g, and provided with food and water *ad libitum*. Mice were randomly allocated into groups, outlined in (Figure 1), with each group containing 4-6 animals.

Femoral Cannulation and Fluid Administration

Mice were anesthetized by inhalation of 5% isoflurane (Attane™, Bomac Pty Ltd., NSW, Australia) at 3 ml/min O_2 titrated to approximately 1.5% isoflurane at 1 L/min O_2. The femoral vein was cannulated and 60 ml/kg body weight of 0.9% saline was manually administered at ~80–100 µl/min over 20 mins to Groups II, IV and VI (Figure 1). Mice were continuously monitored under anesthetic for 10, 40 or 70 mins following the completion of fluid administration. After monitoring, mice were killed via titration of isoflurane to 5% at 3 L/min O_2.

Tissue Resection and Assessing Oedema

The lungs and heart were removed *en bloc* and the upper right lobe was resected, weighed and lyophilized (Freeze Dryer Alpha 2-4LD Plus, John Morris Group) to determine lung wet-to-dry (W/D) weight ratio.

A Bronchoalveolar Lavage (BAL) was performed on the remaining lung with three separate 32 ml/kg body weight volumes of 0.9% sodium chloride at 4°C each instilled and withdrawn three times, before centrifugation (626 g, 10 min, 4°C). The resultant cell pellet was utilized for determination of cell numbers by trypan blue exclusion light microscopy.

The brain, liver, left kidney and gastrocnemius muscle were resected and weighed then freeze dried (Freeze Dryer Alpha 2-4LD Plus, John Morris Group) and again weighed for determination of W/D weight ratio.

Histology Analysis

The remaining lung tissue was fixed in 10% buffered formalin. Paraffin-embedded sections (4 µm) were stained with hematoxylin and eosin. Histology images were taken on an Upright BX63 microscope utilizing a DP73 dual mode color/monochrome camera and cellSens Dimensions v1.16 software (Olympus Life Science Pty Ltd, Australia). Pulmonary inflammatory cell infiltrate and alveolar wall thickening was assessed with a semi-quantitative score (0-3) on blinded sections by two independent investigators [13].

Statistics

Statistical analyses were performed using IBM SPSS Statistics 21.0 software (IBM Corporation, Armonk, NY). All values are expressed as mean ±SE. Two-way analysis of variance (ANOVA) with Bonferroni post hoc tests were used to determine significant differences ($p \leq 0.05$).

Results

Lung tissue demonstrated pulmonary oedema by 30 mins following administration of 60 ml/kg 0.9% saline bolus (treatment) when compared to control, which was sustained to 60 min and had resolved by 90 mins post infusion. There was no effect of time or interaction between treatment and time. (Figure 2A); treatment p=0.003, time p=0.211, treatment*time p=0.112.

Lung cellular infiltrate, measured both in BAL and histologically, increased by 60 min following administration of 60 ml/kg 0.9% saline bolus (treatment) when compared to control. This difference was maintained through 90 mins post infusion, however cell number

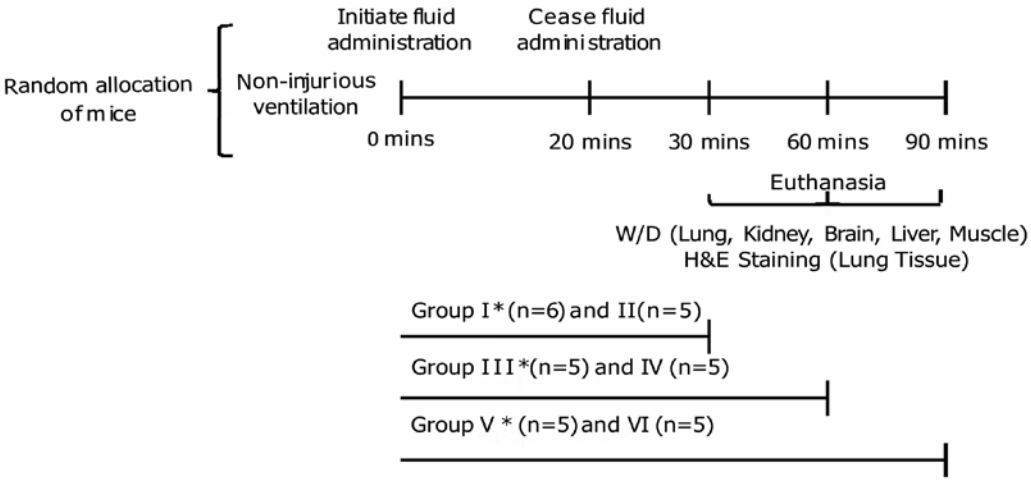

Figure 1: Protocol utilized for fluid administration. Groups I/II represents the 30 mins exposure time point to intravenous fluids, III/IV the 1 h time point, and V/VI the 1.5 h time point. *Denotes control group in which no fluid is administered.

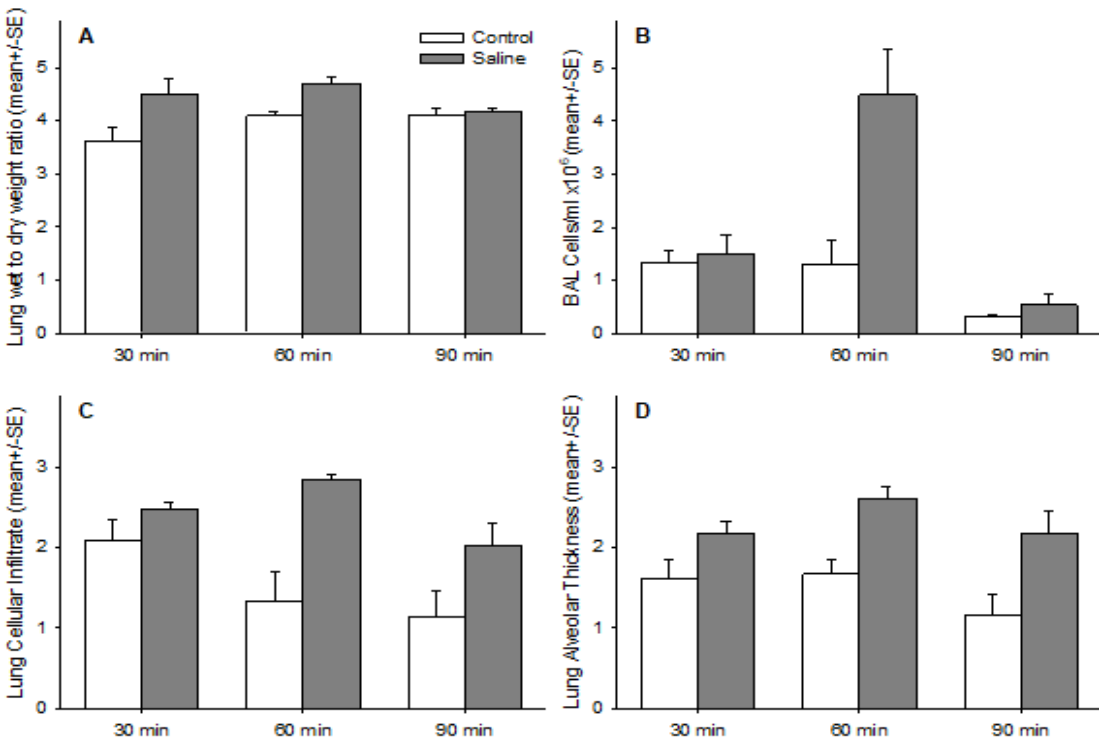

Figure 2: The temporal course of pulmonary injury in C57BL/6 mice 30 mins, 1 h and 1.5 h following IV 0.9% saline administration n=5-6 per group. All data presented as mean ±SE and analyzed by Two-way analysis of variance (ANOVA) with Bonferroni post hoc analysis. **A** Lung wet-to-dry weight (W/D) ratio; treatment p=0.003, time p=0.211, treatment*time p=0.112. **B** Bronchoalveolar Lavage (BAL) cellular infiltrate; treatment p=0.022, time p=0.026, treatment*time p=0.119. Lung histology scores of **C** Cellular Infiltrate (CI); treatment p≤0.001, time p=0.025, treatment*time p=0.128, and **D** Alveolar wall thickness (AT); treatment p≤0.001, time p=0.133, treatment*time p=0.569.

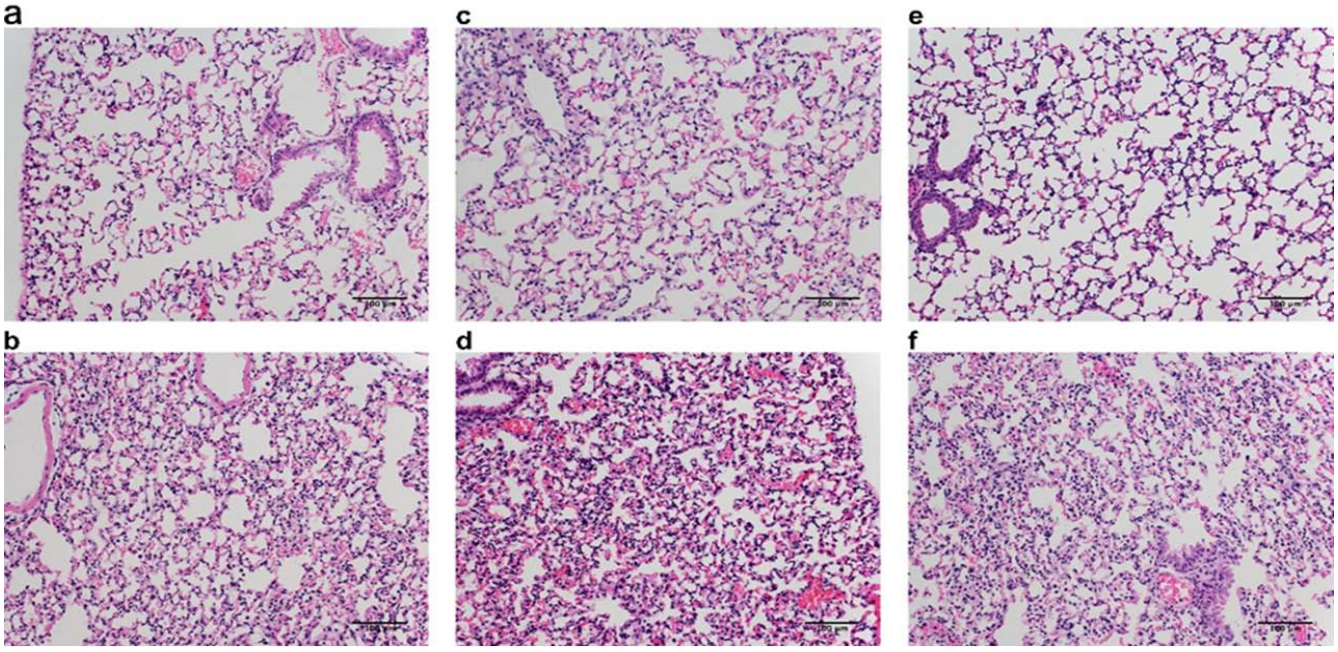

Figure 3: Visual representation of H&E stained lung tissue over the temporal course of FILI following fluid administration. Images represent groups of n=4-6. All images were taken at 20X magnification with scale bars representing 100μm. **A&B** Represents 30 mins control and saline group, respectively. **C&D** Represents 1 h control and saline group, respectively. **E&F** Represents 1.5 h control and saline group, respectively.

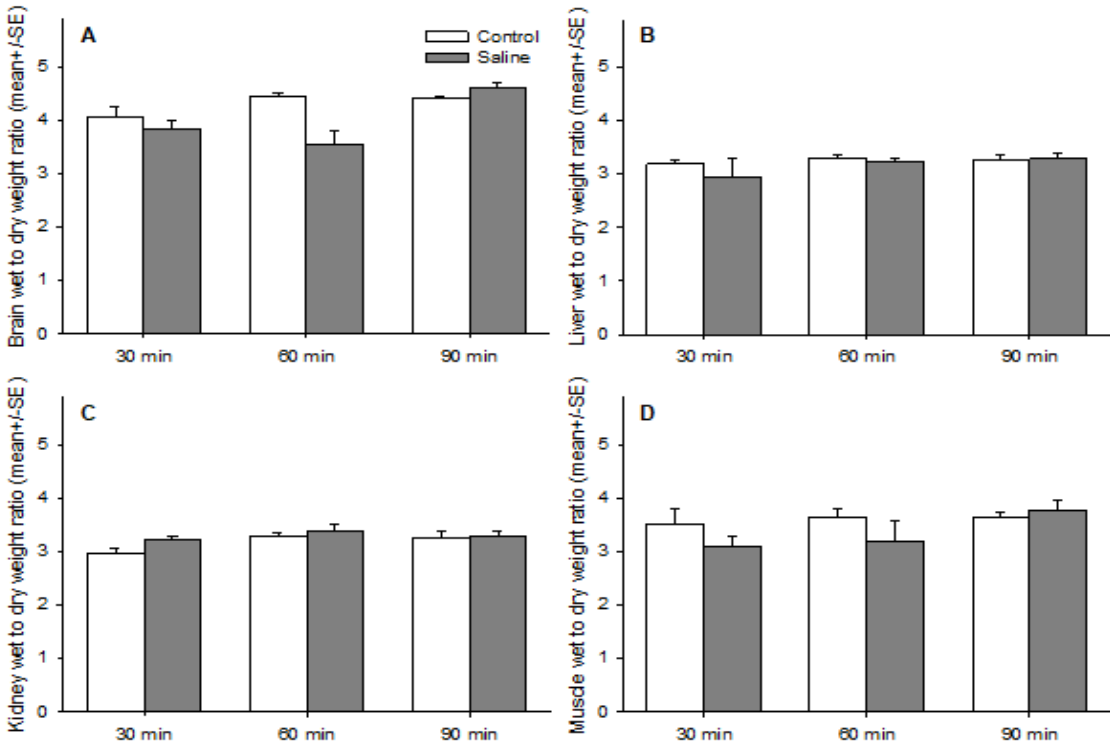

Figure 4: The temporal course of tissue wet-to-dry weight (W/D) ratios in C57BL/6 mice 30 mins, 1 h and 1.5 h following IV 0.9% saline administration n=5-6 per group. All data presented as mean ±SE and analyzed by Two-way analysis of variance (ANOVA) with Bonferroni post hoc analysis. **A** Brain W/D ratio; treatment p=0.035, time p=0.004, treatment*time p=0.013. **B** Liver W/D ratio; treatment p=0.526, time p=0.385, treatment*time p=0.760. **C** Kidney W/D ratio; treatment p=0.108, time p=0.037, treatment*time p=0.453. **D** Muscle W/D ratio; treatment p=0.209, time p=0.238, treatment*time p=0.395.

in both the control and saline treated mice declined suggestive of significant cell clearance in all animals during 90 mins of ventilation, manifesting as a significant time effect. There was no interaction effect between treatment and time for either parameter. BAL cellular infiltrate, (Figure 2B); treatment p=0.022, time p=0.026, treatment*time p=0.119 and histological cellular infiltrate, (Figure 2C); treatment p≤0.001, time p=0.025, treatment*time p=0.128 and (Figure 3 A-F).

Similarly, lung alveolar thickness increased by 60 min following administration of 60 ml/kg 0.9% saline bolus (treatment) when compared to control and this difference was maintained through 90 mins post infusion. There was no effect of time or interaction between treatment and time. (Figure 2D); treatment p≤0.001, time p=0.133, treatment*time p=0.569 and (Figure 3 A-F).

Brain tissue demonstrated fluid loss by 60 mins following administration of 60 ml/kg 0.9% saline bolus (treatment) when compared to control, which was resolved by 90 min post infusion, manifesting in both a time and treatment, time interaction effect. (Figure 4A); treatment p=0.035, time p=0.004, treatment*time p=0.013.

Liver, kidney, and muscle did not show any significant change in tissue hydration following administration of 60 ml/kg 0.9% saline bolus when compared to controls, at any time. Liver, Figure 4B; treatment p=0.526, time p=0.385, treatment*time p=0.760. Kidney, Figure 4C; treatment p=0.108, time p=0.037, treatment*time p=0.453. Muscle, Figure 4D; treatment p=0.209, time p=0.238, treatment*time p=0.395.

Discussion

This study elucidates the development and resolution of fluid induced pulmonary edemain a murine model following rapid infusion of 0.9% saline at 60 ml/kg body weight. We have previously established that Fluid Induced Lung Injury (FILI) with proteinaceous oedema develops 30 mins after initiating fluid administration at 60 ml/kg body weight in a Sprague Dawley rat model [5]. In conducting this study, total exposure time was extended to 1 h and 1.5 h in order to examine the temporal manifestation of fluid induced pulmonary edema. It was evident that pulmonary oedema remains present 1 h after initiating fluid administration. This is consistent with previously reported lung injury and fluid induced pulmonary edema development in rat models and healthy human volunteers [5,11,12]. These results demonstrate the development of fluid induced pulmonary edemain multiple species, which is likely to be facilitated by increased endothelial permeability allowing influx of fluids into the alveolar space.

Both BAL and histological Cellular Infiltrate (CI) as well as Alveolar wall thickness (AT) demonstrated injury at 1 h exposure, consistent with previous reports in rat models [5], but not at 30 mins. In acute lung injury, proteinaceous oedema proceeds accumulation of cellular infiltrate, epithelial barrier proteins and fibrosis [14-16]. This is consistent with our results in which oedema developed before significant increases in CI and AT were observed. At 1.5 h exposure, CI and AT resolved in parallel with resolution of oedema.

Increases in vascular flow induce shear stress and facilitate elevated endothelial permeability [17], therefore allowing influx of fluid into the intracellular space. At 90 mins exposure, FILI in C57BL/6 mice resolved, in contrast to our previous FILI models, where no resolution of injury was observed up to 120 mins [5]. Endothelial permeability is bi-directional, returning to basal levels after return to normal vascular flow [17], providing an explanation for the resolution of FILI. The exact mechanism of fluid clearance is unknown, however aquaporin, ENaC, CFTR and Na^+/K^+ ATPase channels are all expressed in the lung and have been reported in the regulation of alveolar fluid clearance [18-25].

As stated, fluid induced pulmonary edema develops quickly with eventual resolution, but the mechanism of this injury is speculative. A promising candidate that has emerged is the Transient Receptor Potential Vanilloid (TPRV) 4 channel. In rat models, FILI is ameliorated following administration of the non-specific TRPV4 inhibitor, Ruthenium Red, prior to 0.9% saline bolus infusion [5]. Furthermore, $TRPV4^{-/-}$ mice do not develop FILI when administered a saline bolus [5].

Previous reports have associated fluid administration with increased TRPV4 expression in kidney tissue and mortality [26-32]. No evidence of kidney oedema was observed in our mice. Other tissues expressing TRPV4 such as the liver, and muscle tissue [33-36] were investigated but also showed no evidence of edematous injury. Overall, the examination of tissue oedema through W/D weight of all tissues indicates a lung tissue specific manifestation of injury in our model. Similar observations using lactate-pyruvate ratios of liver and kidney tissue in an ovine model of fluid resuscitation provides additional evidence that these tissues are unaffected following fluid infusion [37].

We found that increasing fluid administration reduced cerebral wet to dry weight ratio up to 90 min post-infusion. Acute hypernatremia, as was demonstrated previously in the rat with the same fluid administration protocol [5], leads to intracellular dehydration and decrease in cell volume, particularly in brain cells, producing shrinking of brain size [38]. Conversely, an association between conservative fluid-management strategy and the development of long-term cognitive impairment in ARDS patients admitted to the ICU has been reported [39]. The effects, both short and long term, of saline bolus administration on the brain therefore warrant further investigation.

A limitation of this study is the use of isoflurane to anaesthetize mice, which was not used in our rat FILI model [5]. It has previously been reported that isoflurane may have protective effects in endotoxin-induced lung injury [40]. Further investigation is therefore required to determine whether activation of one fluid channel, co-activation of multiple fluid channels, or the use of isoflurane contributed to FILI resolution in these mice.

In conclusion, the rapid infusion of intravenous fluids demonstrated a temporal course of FILI development and resolution following fluid administration of 0.9% saline bolus at 60 ml/kg body weight in our mouse model, which is not apparent in rats and healthy human volunteers over the same time period [5]. Elucidating the pathway of recovery via highly expressed fluid channels such as aquaporins, ENaC, CFTR, or Na^+/K^+ATPase may provide crucial information for drug targeting which could promote fluid clearance in patients presenting with lung oedema, and subsequently decrease mortality rates. These results are consistent with other models of lung injury following fluid administration and provide additional evidence which may elicit further scrutiny of current fluid resuscitation practices, particularly in critical care environments.

Conflict of Interest

The authors report no conflict of interest.

Acknowledgements

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