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The effect of matrix metalloproteinase-3 deficiency on pulmonary surfactant in a mouse model of acute lung injury

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Abstract

The acute respiratory distress syndrome (ARDS) is characterized by arterial hypoxemia accompanied by severe inflammation and alterations to the pulmonary surfactant system. Published data has demonstrated a protective effect of matrix metalloproteinase-3 (Mmp3) deficiency against the inflammatory response associated with ARDS; however, the effect of Mmp3 on physiologic parameters and alterations to surfactant have not been previously studied. It was hypothesized that Mmp3 deficient (Mmp3^{-/-}) mice would be protected against lung dysfunction associated with ARDS and maintain a functional pulmonary surfactant system. Wild type (WT) and Mmp3^{-/-} mice were subjected to acid-aspiration followed by mechanical ventilation. Mmp3^{-/-} mice maintained higher arterial oxygenation compared with WT mice at the completion of ventilation. Significant increase in functional large aggregate surfactant forms were observed in Mmp3^{-/-} mice compared with WT mice. These findings further support a role of Mmp3 as an attractive therapeutic target for drug development in the setting of ARDS.

Keywords

matrix metalloproteinase-3; acute respiratory distress syndrome; acute lung injury; pulmonary surfactant; mechanical ventilation; acid aspiration; hypoxemia

Introduction

The acute respiratory distress syndrome (ARDS) is characterized by severe lung dysfunction defined by a decrease in the partial pressure of arterial of O₂ (PO₂) to the fractional inspired O₂ (FiO₂) ratio after an acute pulmonary insult (Ranieri et al. 2012). ARDS can occur as a result of a direct insult, such as gastric aspiration, while the effects of mechanical ventilation that are necessary to support the subsequent lung dysfunction have been shown to secondarily exacerbate and accelerate the disease process (Livingston et al. 1995; Slutsky and Ranieri 2000). Importantly, there are no proven pharmacological therapies while the mortality associated with ARDS continues to exceed 30% (Bosma et al. 2010). Further studies on molecular targets that influence the progression of ARDS are required in order to develop potential new pharmacological therapies.

Despite a physiologic clinical definition, the underlying pathophysiological processes involved in ARDS are complex and include the development of overwhelming pulmonary inflammation, leakage of protein-rich edema into the distal airspaces, and alterations to the pulmonary surfactant system (Ware and Matthay 2000). The changes to the pulmonary surfactant system have been of therapeutic interest, as the known biophysical properties of surfactant enhance lung compliance and promote arterial oxygenation (Goerke 1998). Impairment of the surfactant system has been well documented in the setting of ARDS and includes a reduction in the relative amount of functional large aggregate (LA), compared with the inactive small aggregates (SA) forms, as well as an impairment in the ability of LAs to reduce or lower surface tension (Frerking et al. 2001).

One of the recent molecular targets identified in the pathogenesis of ARDS in animal models is matrix metalloproteinase-3 (Mmp3). This matrix metalloproteinase belongs to a family of zinc-dependent proteases that has important roles in innate immunity and inflammation (Nissinen and Kähäri 2014; Senior and Shapiro 1992). For example, it has been observed that mice lacking Mmp3 were protected against the development of pulmonary inflammation in 2 distinct models of ARDS. (Nerusu et al. 2007; Warner et al. 2001). From a clinical perspective, elevated levels of Mmp3 in lavage fluid of patients with ARDS have been shown to correlate directly with mortality and the development of multi-organ failure (Fligiel et al. 2006). To our knowledge, the effects of Mmp3 on physiologic changes observed in ARDS and its effects on the host pulmonary surfactant system have not been reported. We hypothesized that mice deficient in Mmp3 would develop a less severe lung injury, which would be associated with a preservation of a more functional surfactant system.

Methods

Animals

All experiments were performed in concordance with Canadian Council of Animal Care and approved by the Animal Use Subcommittee of Western University. C57BL/6 wild type (WT) and Mmp3 deficient (Mmp3^{-/-}) (Taconic, Hudson, New York, USA) female mice (10–16 weeks old, mass 20–25 g) were used for all experiments. Mice were bred and housed in our laboratory and had unlimited access to food and water.

Injury model

Details of this model have been previously reported (Yamashita et al. 2014). Briefly, WT and Mmp3^{-/-} mice were anesthetized and instrumented with vascular lines and connected to a ventilator (Harvard Apparatus, Massachusetts, USA) via an endotracheal tube and ventilated with the following parameters: respiratory rate = 150 breaths/min; tidal volume = 10 mL/kg; positive end expiratory pressure (PEEP) = 3 cm H₂O; and FiO₂ = 1.0; and baseline physiological values were recorded. Subsequently, animals were randomized to receive an intra-tracheal bolus of air or 50 µL of 0.05 mol/L hydrochloric acid (HCl, pH 1.10), resulting in 4 experimental groups: (1) WT air, (2) Mmp3^{-/-} air, (3) WT acid, and (4) Mmp3^{-/-} acid. Animals were ventilated for 240 min. Physiological measurements were taken at 120 and 240 min. Following mechanical ventilation, mice were sacrificed (sodium pentobarbital, 110 mg/kg) and whole lung lavage was performed.

Lung lavage and surfactant analysis

Whole lung lavage and surfactant isolation was performed as previously described (Yamashita et al. 2014). To determine surfactant pool sizes, samples were extracted using the Bligh and Dyer method and quantified using the Duck-Chong phosphorous assay (Bligh and Dyer 1959; Duck-Chong 1979; Yamashita et al. 2014). Surfactant activity was measured using a constrained sessile drop surfactometer (CDS; BioSurface Instruments, Hawaii, USA) (Valle et al. 2015). Briefly, a 9 µL drop of LA sample at 1 mg phospholipid/mL was placed on the instrument pedestal. The drop was cyclically compressed–expanded for 25 cycles at a rate of 1 cycle/s, and a compression of 22% ± 2% using a computer controlled external stepper motor. Images were recorded at a rate of 10 images/s, and were used to determine the surface tension in conjunction with axisymmetric drop shape analysis. Minimum surface tension at cycles 1, 5, and 25 was used as an indication of biophysical function.

Statistical analysis

All data are expressed as mean ± SEM. Statistical analysis was performed using statistical software package GraphPad Prism 5 (GraphPad Software, La Jolla, California, USA). A two-way ANOVA was used to explore any interactive effects followed by a one-way ANOVA with Tukey's post hoc test for pairwise comparison of groups. *P* values less than 0.05 were considered statistically significant.

Results

The arterial oxygenation over 240 min of mechanical ventilation of WT and *Mmp3*^{-/-} mice exposed to acid aspiration and baseline controls is shown in Fig. 1. At baseline, no significant differences were observed in arterial oxygenation among the 4 experimental groups. Statistical comparison shows that after 120 and 240 min of mechanical ventilation, PaO₂/FiO₂ ratios were significantly lower than those observed at baseline for all experimental groups. At 120 min, the oxygenation values were not significantly different among the 4 groups. However, at the completion of mechanical ventilation, WT mice randomized to acid aspiration had a significantly lower PaO₂/FiO₂ ratio compared with WT mice receiving a control air bolus at the same time point. In contrast, *Mmp3*^{-/-} mice randomized to acid aspiration did not show a significant difference in the PaO₂/FiO₂ ratio compared with air-instilled animals. Furthermore, comparison of WT and *Mmp3*^{-/-} mice revealed a significant difference in PaO₂/FiO₂ between acid-instilled WT and *Mmp3*^{-/-} mice at 240 min.

Values of other physiological parameters measured during mechanical ventilation are shown in Table 1. Overall, values of PCO₂, PIP, and heart rate were not significantly different among the 4 groups. The PCO₂ values increased during ventilation in all groups and, similarly, PIP increased during ventilation in all experimental groups. A trend toward higher PIP values was observed in animals receiving acid instillation; however, these differences did not reach statistical significance. Significant differences were observed in PIP values measured at earlier time points (i.e., 90 min) in animals receiving acid aspiration compared with air controls; however, these differences did not persist for the remainder of the mechanical ventilation protocol (data not shown).

Results from the analysis of surfactant pools and biophysical activity are depicted in Fig. 2 and Table 2, respectively. The total amount of surfactant measured in the lavage fluid was not significantly different among the 4 groups (Fig. 2A). Significant differences in the amount of LA were observed between groups (Fig. 2B). Specifically, WT mice randomized to acid aspiration had lower amounts of LA compared with WT air-instilled mice at the completion of the mechanical ventilation, whereas no differences in LA pools were observed between acid-instilled and air-instilled *Mmp3*^{-/-} animals. The amount of (SA) was elevated in the WT acid group, and did not reach statistical significance (Fig. 2C). Expression of the aggregate pool sizes as a percentage of LA (Fig. 2D) also revealed a significant decrease in WT acid versus WT air-instilled mice, with no significant differences observed between the 2 *Mmp3*^{-/-} groups. Analysis of the surface activity, expressed as minimum surface tension of the LA fractions, during dynamic compression cycles showed low surface tension values for all samples with no significant differences among groups (Table 2).

Discussion

In the current study, it was hypothesized that *Mmp3*^{-/-} mice would be protected against the development of the physiologic dysfunction associated severe lung injury and this would be associated with a more functional surfactant system. In general, our data supported this hypothesis, as *Mmp3*^{-/-} mice exposed to acid injury followed by ventilation maintained

higher PO_2/FiO_2 values compared with $Mmp3^{+/+}$. In addition, WT mice demonstrated a decrease in the relative percentage of LA in the lavage typically seen in the setting of ARDS, whereas this change was not observed in the $Mmp3^{-/-}$ mice. We conclude that the deficiency in *Mmp3* is protective against the physiologic changes observed in lung injury, and this is, in part, due to maintenance of functional surfactant system.

Whereas previous studies have focused on the inflammatory effects of *Mmp3* deficiency in the context of ARDS (Nerusu et al. 2007; Warner et al. 2001), the current study addressed the potential effects of *Mmp3* deficiency on the pulmonary surfactant system in a relevant animal model of lung injury. The focus of the current study was based on the 2 relevant observations. Firstly, in the context of ARDS, alteration to surfactant represents one of the fundamental deleterious processes contributing to decreased oxygenation (Frerking et al. 2001) and, secondly, protease activity has been shown to alter surfactant metabolism and function (Gross 1995; Malloy et al. 2005). For example, Gross has shown that a specific protease, convertase, could enhance the conversion of LA into SA surfactant sub-fractions in vitro (Gross 1995). Furthermore, our lab has shown that other proteases could also propagate this LA to SA conversion and that this process was mediated by the degradation of either surfactant-associated protein A (SP-A), or SP-B (Veldhuizen et al. 1994). Preliminary experiments in our lab indicated that purified *Mmp3* could also degrade purified SP-A; however, this effect was mitigated when the SP-A was incubated together with surfactant lipids (data not shown). It is, therefore, notable in that $Mmp3^{-/-}$ mice exhibited maintenance of LA pool sizes compared with WT mice when exposed to lung injury resulting from acid aspiration with mechanical ventilation. This maintenance of LA pool sizes was consistent with preservation of arterial oxygenation at the completion of the study. It is tempting to speculate that the maintenance of LA pool sizes can be attributable to the lack of *Mmp3*; however, due to the complex biological pathways of surfactant metabolism and synthesis, other mechanisms that could contribute to this observation would require further studies.

In addition to pool sizes, we also analyzed surfactant function using a constrained sessile drop surfactometer (Zuo et al. 2008). This technique represents an ideal method for analysis of mouse samples as it allows for rapid determination of surfactant function with small sample volumes. The low surface tension achievable in each of the experimental groups, indicative of preserved surfactant function, would appear contrary to the assessment of surfactant function described previously in patients with ARDS or experimental animal models of ARDS (Frerking et al. 2001). The model used in the current study, although representative of ARDS via acid aspiration followed by mechanical ventilation, represented a relatively minor and early stage of lung injury based on measurements of several physiological parameters. Because surfactant function is dependent on both its inherent surface activity as well as its pool size, the data suggest that a decrease in pool sizes may be the main mechanism by which alteration of surfactant could influence the lung.

Overall, several lines of published evidence support a role of *Mmp3* in contributing toward the pathogenesis of ARDS (Fligiel et al. 2006; Nerusu et al. 2007; Warner et al. 2001). For example, previous studies have demonstrated a protective effect of *Mmp3* deficiency against inflammatory responses in several experimental models of acute lung injury (Nerusu et al. 2007; Warner et al. 2001). In addition, increased levels of *Mmp3* levels in lung lavage

material from patient with ARDS correlated with disease severity, incidence of multi-organ failure, and mortality (Fligiel et al. 2006). The current study adds to the current state of the literature by providing evidence for the protective effect of Mmp3 deficiency in a clinically relevant model of lung injury through maintenance of functional surfactant pool sizes. Despite some limitations of our study, such as (1) potential indirect effects of Mmp3 deficiency on alterations in other MMPs or TIMP activity, (2) the clinical relevance of short-term experimental models of ARDS, or (3) uniform, weight-independent volumes of acid delivered, the combination of our results with current literature provides a strong rationale for further studies examining the potential role of Mmp3 as a therapeutic target in ARDS.

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References

- Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* 1959; 37:911–917. DOI: 10.1139/o59-099 [PubMed: 13671378]
- Bosma KJ, Taneja R, Lewis JF. Pharmacotherapy for prevention and treatment of acute respiratory distress syndrome: current and experimental approaches. *Drugs.* 2010; 70(10):1255–1282. DOI: 10.2165/10898570-000000000-00000 [PubMed: 20568833]
- Duck-Chong CG. A rapid sensitive method for determining phospholipid phosphorus involving digestion with magnesium nitrate. *Lipids.* 1979; 14:492–497. DOI: 10.1007/BF02533467
- Fligiel SEG, Standiford T, Fligiel HM, Tashkin D, Strieter RM, Warner RL, et al. Matrix metalloproteinases and matrix metalloproteinase inhibitors in acute lung injury. *Hum Pathol.* 2006; 37(4):422–430. DOI: 10.1016/j.humpath.2005.11.023 [PubMed: 16564916]
- Frerking I, Günther A, Seeger W, Pison U. Pulmonary surfactant: functions, abnormalities and therapeutic options. *Intens Care Med.* 2001; 27(11):1699–1717. DOI: 10.1007/s00134-001-1121-5
- Goerke J. Pulmonary surfactant: functions and molecular composition. *Biochim Biophys Acta.* 1998; 1408(2–3):79–89. [PubMed: 9813251]
- Gross NJ. Extracellular metabolism of pulmonary surfactant: the role of a new serine protease. *Annu Rev Physiol.* 1995; 57:135–150. DOI: 10.1146/annurev.ph.57.030195.001031 [PubMed: 7778861]
- Livingston, DH., Mosenthal, AC., Deitch, EA. Sepsis and multiple organ dysfunction syndrome: a clinical- mechanistic overview. *New Horizons*; Baltimore, MD: 1995. p. 257-264.
- Malloy JL, Veldhuizen RAW, Thibodeaux BA, O'Callaghan RJ, Wright JR. Pseudomonas aeruginosa protease IV degrades surfactant proteins and inhibits surfactant host defense and biophysical functions. *Am J Physiol Lung Cell and Mol Physiol.* 2005; 288(2):L409–L418. [PubMed: 15516485]
- Nerusu KC, Warner RL, Bhagavathula N, McClintock SD, Johnson KJ, Varani J. Matrix metalloproteinase-3 (stromelysin-1) in acute inflammatory tissue injury. *Ex Mol Pathol.* 2007; 83(2):169–176. DOI: 10.1016/j.yexmp.2007.04.003
- Nissinen L, Kähäri VM. Matrix metalloproteinases in inflammation. *Biochim Biophys Acta.* 2014; 1840(8):2571–2580. DOI: 10.1016/j.bbagen.2014.03.007 [PubMed: 24631662]
- Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. ARDS Definition Task Force. Acute respiratory distress syndrome: the Berlin Definition. *JAMA.* 2012; 307(23):2526–2533. DOI: 10.1001/jama.2012.5669 [PubMed: 22797452]
- Senior RM, Shapiro SD. Introduction: the matrix metalloproteinase family. *Am J Respir Cell Mol Biol.* 1992; 7(2):119.doi: 10.1165/ajrcmb/7.2.119 [PubMed: 1497899]
- Slutsky AS, Ranieri VM. Mechanical ventilation: lessons from the ARDSNet trial. *Respir Res.* 2000; 1(2):73–77. DOI: 10.1186/rr15 [PubMed: 11667968]

- Valle RP, Wu T, Zuo YY. Biophysical influence of airborne carbon nanomaterials on natural pulmonary surfactant. *ACS Nano*. 2015; 9(5):5413–5421. DOI: 10.1021/acsnano.5b01181 [PubMed: 25929264]
- Veldhuizen RA, Hearn SA, Lewis JF, Possmayer F. Surface-area cycling of different surfactant preparations: SP-A and SP-B are essential for large-aggregate integrity. *Biochem J*. 1994; 300:519–524. DOI: 10.1042/bj3000519 [PubMed: 8002958]
- Ware LB, Matthay MA. The acute respiratory distress syndrome. *New Engl J Med*. 2000; 342(18): 1334–1349. DOI: 10.1056/NEJM200005043421806 [PubMed: 10793167]
- Warner RL, Beltran L, Younkin EM, Lewis CS, Weiss SJ, Varani J, Johnson KJ. Role of stromelysin 1 and gelatinase B in experimental acute lung injury. *Am J Respir Cell Mol Biol*. 2001; 24(5):537–544. DOI: 10.1165/ajrcmb.24.5.4160 [PubMed: 11350822]
- Yamashita CM, Fessler MB, Vasanthamohan L, Lac J, Madenspacher J, McCaig L, et al. Apolipoprotein E-deficient mice are susceptible to the development of acute lung injury. *Respiration*. 2014; 87(5):416–427. DOI: 10.1159/000358438 [PubMed: 24662316]
- Zuo YY, Veldhuizen RAW, Neumann AW, Petersen NO, Possmayer F. Current perspectives in pulmonary surfactant — inhibition, enhancement and evaluation. *Biochim Biophys Acta*. 2008; 1778(10):1947–1977. DOI: 10.1016/j.bbame.2008.03.021 [PubMed: 18433715]

Fig. 1.

Oxygenation values over time. Arterial blood gas samples were analyzed to determine the PO_2/FiO_2 before randomization (Baseline) and after 120 and 240 min of mechanical ventilation. $n = 6-7$ animals per group. *, $P < 0.05$ versus wild type (WT) air; #, $P < 0.05$ versus WT acid. Mmp3^{-/-}, Mmp3 deficient.

Fig. 2.

Values of (A) total surfactant, (B) large aggregates (LA), (C) small aggregates (SA), and (D) percent LA as recovered from lung lavage in the 4 experimental groups. $n = 5-6$ animals per group. *, $P < 0.05$ versus wild type (WT) air. Mmp3^{-/-}, Mmp3 deficient.

Table 1

Arterial partial pressure of carbon dioxide (PCO₂), peak inspired pressure (PIP), and heart rate (HR) values at different time points during mechanical ventilation for wild type (WT) and Mmp3 deficient (Mmp3^{-/-}) mice randomized to an intra-tracheal bolus of air or acid followed by 240 minutes of mechanical ventilation.

	Air		Acid	
	WT	Mmp3 ^{-/-}	WT	Mmp3 ^{-/-}
PCO₂ (mmHg)				
Baseline	39.4±2.7	45.2±3.1	41.2±1.4	46.3±4.8
120 min	51.0±2.8	54.8±3.2	59.5±2.8	64.9±6.1
240 min	53.9±3.6	48.3±3.2	60.4±4.9	62.4±6.7
PIP (cm H₂O)				
Baseline	8.4±0.2	9.6±0.5	8.0±0.3	8.3±0.2
120 min	11.7±0.2	12.6±0.6	13.5±0.2	13.1±0.5
240 min	12.7±0.4	12.9±0.8	14.2±0.2	13.6±0.5
HR (beats/min)				
Baseline	285±29	298±26	263±30	288±31
120 min	239±35	262±26	244±18	235±24
240 min	263±30	263±33	242±25	274±30

Note: Values are expressed as means ± SEM, *n* = 4–6.

Table 2

Minimum surface tension of isolated surfactant large aggregates over various dynamic compression–expansion cycles.

Minimum surface tension (mN/m)	<u>Air</u>		<u>Acid</u>	
	WT	Mmp3 ^{-/-}	WT	Mmp3 ^{-/-}
Cycle 1	3.9±0.6	6.1±0.9	5.2±0.8	6.5±0.6
Cycle 5	4.0±0.3	4.5±0.4	4.4±0.8	4.2±0.7
Cycle 25	3.8±0.5	3.2±0.2	3.3±0.6	3.3±0.6

Note: Values are expressed as means ± SEM, $n = 4-5$. There were no significant differences among groups. Mmp3^{-/-}, Mmp3 deficient; WT, wild type.