ORIGINAL RESEARCH

Chantal Massé, BSc^{1,3}

Yves Berthiaume, MD, MSc, FRCPC^{1,2,3}

Effect of adrenalectomy on pulmonary edema resolution

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¹ Centre de recherche, Centre hospitalier de
l'Université de Montréal, Montréal, QC

² Département de médecine, Université de
Montréal, Montréal, QC

³ Institut de recherches cliniques de Montréal,
Montréal, QC

Abstract

Purpose: The capacity of the lung to clear edema fluid has been shown to be one of the factors that can influence the prognosis of cardiogenic and noncardiogenic pulmonary edema. Active Na⁺ transport across the alveolar epithelium is the main driving force involved in this physiological process. Since endogenous catecholamines are known to activate the sodium-dependent mechanism of alveolar edema clearance, the objective of the present study was to explore if adrenalectomy, which prevents the release of endogenous catecholamines and other hormones, such as corticosterone, into circulation, would affect edema resolution in a model of lung injury induced by thiourea.

Methods: A high-permeability pulmonary edema was induced in adult male Sprague-Dawley rats using a thiourea-induced pulmonary edema model. To determine if the release of adrenalin and corticosterone is essential for resolution of the thiourea-induced edema, we measured 1) the release of adrenalin and corticosterone in urine and 2) edema resolution in control animals and adrenalectomized animals.

Results: The administration of thiourea significantly increased the wet-to-dry ratio after four and eight hours. After 12 and 24 hours, the wet-to-dry ratio gradually returned to baseline. Although thiourea-induced pulmonary edema was associated with a significant increase in urine adrenalin and corticosterone, the absence of adrenalin and corticosterone response in adrenalectomized animals did not prevent the resolution of the edema.

Conclusions: These experiments demonstrated that resolution of thiourea-induced pulmonary edema can occur in the absence of hormonal secretion by the adrenal glands.

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Correspondence to:

Dr. Yves Berthiaume

Email: yves.berthiaume@umontreal.ca

The capacity of the lung to clear fluid is one of the factors that can influence the prognosis of cardiogenic and noncardiogenic pulmonary edema [1-3]. Active Na⁺ transport is the main driving force of the physiological process leading to edema clearance from the alveolar space. The epithelial sodium channel (ENaC) at the apical surface and the sodium–potassium adenosine triphosphatase (Na⁺-K⁺-ATPase) at the basolateral surface of alveolar epithelial cells are two essential components of this alveolar liquid clearance, as they create an osmotic gradient that leads to transalveolar liquid transport [4-6]. Several compounds have been shown to stimulate this sodium-dependent alveolar liquid clearance mechanism [7-9].

Endogenous catecholamines released from the adrenal glands have also been proposed as a triggering mechanism of alveolar liquid clearance in different animal models [10-15]. Their potential role is also suggested in experiments in which the overexpression of the β -adrenergic receptor is associated with increased edema clearance [16]; however, there are few studies that have reported the impact of endogenous hormone release by the adrenal glands on edema resolution in models of lung injury [10,11,15]. Furthermore, in those models, there was either no increase in the wet-to-dry ratio of the lungs [10,15] or a minimal increase that was not associated with alveolar edema [11]. The objective of the present study was to explore if adrenalectomy, which prevents the release of endogenous catecholamines and corticosterone into circulation, would affect edema resolution in a model of lung injury induced by thiourea where there is a minimal amount of alveolar edema [17-19].

Materials and methods

Animal models

Thiourea model of lung edema and edema measurement: All animal experiments were approved by the institutional animal care committee of the Research Centre of the Centre hospitalier de l'Université de Montréal and were conducted in accordance with the Principles of Laboratory Animal Care formulated by the Institute of Laboratory Animal Resources and the Guide for the Care and Use of Laboratory (National Institutes of Health Publication No. 86-23, revised 1985). High-permeability pulmonary edema was induced in adult male Sprague-Dawley rats (weight 300–350 g) (Charles River Canada, St. Constant, QC) by intraperitoneal injection of thiourea (15 mg/kg) dissolved in sterile normal saline. The controls received an equivalent volume of normal saline.

After 4, 8, 12 or 24 hours, the animals were anesthetized with pentobarbital sodium (60 mg/kg intraperitoneal) and exsanguinated via transection of the abdominal aorta and inferior vena cava. A blood sample was taken to measure the wet-to-dry ratio. The lungs and mediastinal structures were then removed *in toto*. The right lung was used for the measurement of the wet-to-dry lung weight ratio, which was used as a quantitative measure of lung edema, as previously described [18].

Adrenalectomy in rats: To determine if the release of adrenalin and corticosterone is essential for thiourea-induced pulmonary edema resolution, the release of adrenalin and corticosterone in urine and edema resolution was measured in control and adrenalectomized animals. The adrenalectomized rats were purchased from Charles-River (Charles River Canada, St. Constant, QC) and supplemented with sodium (0.9% saline solution as drinking water) for one week before being included in the experimental protocol.

Urinary adrenalin and corticosterone: To study the stress response during thiourea-induced pulmonary edema, we measured the release of adrenalin and corticosterone in the urine four hours and eight hours after animals received thiourea or normal saline and after eight hours in adrenalectomized animals. The urine from animals kept in metabolic cages was continuously collected and kept on ice. Samples of the urine collected were used to measure adrenalin and corticosterone. The results were normalized to the concentration of creatinine in the urine. Urine was acidified with 10 μ l of HCl 6 N/ml of urine, and adrenalin was measured by high pressure liquid chromatography using a technique similar to one used previously [20]. Corticosterone was measured by radioimmunoassay (Coat-a-count, Rat Corticosterone, no cat: TKRC-1, Diagnostic Products Corporation, Los Angeles, USA).

Data analysis

The data are presented as the mean $\pm$ the standard error of the mean (SEM) in all figures. A two-way analysis of variance, followed by Bonferroni *post-hoc* test, was used to determine if there was a significant difference between the wet-to-dry ratio found in the thiourea-treated animals and the control animals as shown in Figures 1 and 2. A t-test was used for the comparison between the wet-to-dry ratio after eight hours and 24 hours (Figure 2). The data in Figure 3 were analyzed with a two-way analysis of variance followed by Bonferroni *post-hoc* test. We accepted $p < 0.05$ as significant.

FIGURE 1.

Measurement of the wet-to-dry ratio at different time points after the injection of normal saline (open bars) or thiourea (black bars): four hours (ctl n=17, th n=17); eight hours (ctl n=14, th n=14); 12 hours (ctl n=9, th n=9); and 24 hours (ctl n=10, th n=10). Abbreviations: ctl=control (normal saline); n=number of animals; th=thiourea; and W/D=wet to dry ratio.

* = $P < 0.05$ between control and thiourea

= $P < 0.05$ between 8 hours and 24 hours for thiourea

FIGURE 2.

Measurement of the wet-to-dry ratio at different time points after the injection of normal saline (open bars) or thiourea (black bars) with or without adrenalectomy (ADX): eight hours without ADX (ctl n=5, th n=5); eight hours with ADX (ctl n=5, th n=5); 24 hours without ADX (ctl n=5, th n=5); and 24 hours with ADX (ctl n=5, th n=5). Abbreviations: ctl=control (normal saline); n=number of animals; th=thiourea; and W/D=wet to dry ratio.

* = $P < 0.05$ between control and thiourea

* # = $P < 0.05$ between 8 hours and 24 hours

Results

The administration of thiourea significantly increased the wet-to-dry ratio four and eight hours after intraperitoneal injection (Figure 1). After 12 and 24 hours, the wet-to-dry ratio gradually returned to baseline (Figure 1). The induction of pulmonary edema with thiourea was associated with a significant increase in urine adrenalin and corticosterone eight hours after injection of thiourea (Figure 3).

In adrenalectomized rats, there was no increase in urinary adrenalin or corticosterone eight hours after the injection of thiourea (Figure 3); however, the increase in the wet-to-dry ratio after eight hours was similar to the increase observed in normal rats (Figure 2). Furthermore, the absence of adrenalin and corticosterone response did not prevent the resolution of edema (Figure 2).

Discussion

The results of these experiments demonstrated that the resolution of pulmonary edema, or at least of thiourea-induced pulmonary edema, can occur in the absence of hormonal secretion by the adrenal glands.

Following the injection of thiourea, the wet-to-dry ratio increased to a value close to six in both control and adrenalectomized animals. Furthermore, the wet-to-dry ratio returned to nearly the preinjection level 24 hours after the injection of thiourea in animals; both those with and without adrenal glands (Figure 2). Since the wet-to-dry ratio observed at four and eight hours was found to be associated with alveolar edema in a previous study [19], our results suggest that the adrenal glands and the hormones they secrete are not necessary to initiate alveolar liquid clearance and edema resolution. The validity of this interpretation depends on the

FIGURE 3.

Measurement of the concentration of adrenalin (A) and corticosterone (B) in urine collected after the injection of normal saline (open bars) or thiourea (black bars) with or without adrenalectomy (ADX): four hours without ADX (ctl n=5, th n=5); eight hours without ADX (ctl n=5, th n=5); and eight hours with ADX (ctl n=4, th n=5). Abbreviations: ctl=control (normal saline); n=number of animals; and th=thiourea.

*= $P < 0.05$ between control and thiourea

demonstration that the adrenalectomy was successful. The significant decrease in both adrenalin and corticosterone in the urine (Figure 3) was consistent with successfully adrenalectomization; however, we cannot exclude the possibility that there were other adaptive measures that could have played a role in triggering edema resolution. Indeed, following adrenalectomy, an increase in antidiuretic hormone and plasma volume expansion have been observed [21]. Large concentrations of antidiuretic hormone have been shown to modulate fetal liquid clearance [22] and could potentially stimulate alveolar liquid clearance in adult lungs, but physiological concentrations induced only a modest response [23]. It is also possible that extra-adrenal tissue synthesis of mineralocorticoids, such as aldosterone or glucocorticoids, can occur [24] and might influence Na^+ transport and edema clearance in the lungs [25]. We did not measure the antidiuretic hormone response or the presence of other mineralocorticoids in our animals, but it seems unlikely that this type of adaptation response would occur, since we provided the animals with salt supplementation,

which has been shown to maintain animal health, normal blood pressure, cardiac output and electrolyte balance [21].

Our results are inconsistent with previous results that showed an important role of endogenous hormones, especially catecholamines, in the modulation of sodium-dependent alveolar liquid clearance. Using different animal models of lung injury, such as sepsis [11], hemorrhagic shock [10], intracranial hypertension [12] and acute pancreatitis [15], several authors have shown that endogenous catecholamines released from the adrenal glands contributed to the activation of active sodium transport and alveolar fluid clearance. These data support the concept that the lungs can respond to endogenous catecholamines, which might help prevent flooding of the airspaces in the early phase of those clinical conditions. Likewise, in the presence of left atrial hypertension, endogenous catecholamines have been shown to maintain alveolar fluid clearance [13]. Finally, in the neonatal period, endogenous release of catecholamines is essential for sodium-dependent clearance of fetal lung liquid [14].

How can the discrepancy between our results and previously published data be explained? First, in some of the previously published manuscripts, the investigators measured alveolar liquid clearance and not the resolution of pulmonary edema [10,11]. Indeed, there was no biologically significant increase in the wet-to-dry ratio in the hemorrhagic shock model (wet-to-dry ~ 3.4) [10] or in the sepsis model (wet-to-dry ~ 4.0) [11]. In these models, alveolar liquid clearance was measured in animals that had been exposed to a significant systemic stress but did not have significant pulmonary edema. It is quite plausible that the mechanisms that trigger edema resolution might be different in the absence of significant edema. Second, in a number of manuscripts where alveolar liquid clearance was studied in adrenalectomized animals [10,12,13], there was always a remaining level of alveolar liquid clearance even in the absence of the adrenal gland. In fact, in the study by Campbell et al. [13], they estimated that the endogenous catecholamines contributed to only 30% of the alveolar liquid clearance that occurred in the presence of left atrial hypertension. Overall, these observations suggest that there must be other biological signals that trigger liquid clearance, even in the absence of circulating catecholamines or other hormones secreted by the adrenal glands.

The present study was not designed to identify what biological factors could modulate edema resolution in the absence of adrenal glands; however, we can speculate on potential molecules that could be involved in this process. Previous studies using the thiourea-induced lung injury model have shown histologically that there was significant edema accumulation in the alveolus and in the interstitial space [17,19]. This accumulation of liquid in the lung could trigger physiological responses known to stimulate lung neuroendocrine cells [26], which can release amines, such as serotonin [26,27] or dopamine [28], which are known to stimulate lung liquid clearance [28-31]. Future experiments are needed to explore this hypothesis.

Clinical studies established that the capacity to remove pulmonary edema was impaired in most patients with lung injury and sepsis-induced acute respiratory distress syndrome, and this impairment correlated with higher mortality [2,32]. Multiple pathophysiological mechanisms were found to be responsible for impaired alveolar fluid clearance and the lack of increase of alveolar fluid clearance following pharmacological stimulation in injured lungs [33-35].

Novel therapies will need to be designed to improve edema clearance in injured lungs. The present study provides new data that show that factors that are probably intrinsic to the lung might trigger a physiological response that initiates edema resolution. Future studies of this mechanism might help us develop novel therapies for pulmonary edema.

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