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SHORT COMMUNICATION

Dexamethasone acutely accelerates pleural fluid absorption in mice hydrothoraces

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Abstract This study assessed the effect of corticosteroid treatment in the clearance of hydrothoraces in mice. Twenty-four C57BL/6 mice were divided into four groups and were injected intrapleurally with 500 μL sterilized PBS-BSA 1% to create isosmotic hydrothoraces. Two groups served as control and two groups were treated with dexamethasone. The control groups received intraperitoneally PBS, while the corticosteroid treatment groups received dexamethasone (1 mg/kg), both 5 min after the induction of hydrothorax. Control and treated animals were sacrificed 2 and 4 h after the induction of hydrothorax, and pleural fluid volume was measured. The pleural fluid volume 2 and 4 h after the induction of hydrothoraces was significantly lower in the dexamethasone-treated group compared to the untreated group. The rate of pleural fluid absorption 2 and 4 h after the induction of hydrothoraces was significantly higher in the dexamethasone-treated groups. The present study demonstrated that dexamethasone accelerates pleural fluid absorption in induced isosmotic hydrothoraces in mice. This newly reported property of dexamethasone may partly account for the clinical observation of faster resolution of pleural effusions when corticosteroids are administered in patients with pleural effusions of certain etiologies.

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Introduction

Corticosteroids are used to treat inflammatory pleural effusions of certain etiologies [1–4]. The actual mechanism through which administration of corticosteroids results in a faster resolution of pleural effusions is not known, but it is attributed to their potent anti-inflammatory effects. In connection to this, dexamethasone has been shown to suppress endotoxin- or pneumothorax-induced pleural inflammation in rodents [5, 6]. It is thus believed that attenuation of the inflammatory response leads to reduction of pleural fluid production.

However, whether corticosteroids affect pleural fluid absorption, the second component of the pleural fluid homeostasis, has never been examined. In this connection, corticosteroids have been reported to up-regulate the expression, activity and translocation to the plasma membrane of ion transporting proteins (i.e. ENaC, Na^+-K^+ ATPase, Na^+/H^+ exchanger) [7–11], which, in turn, have been implicated in pleural fluid absorption [12, 13]. We thus hypothesized that corticosteroids increase the absorption rate of isosmotic hydrothoraces in mice.

Methods

Mouse model

Animal experiments were approved by the Veterinary Administration Bureau of the Prefecture of Athens, Greece. Wild-type C57BL/6 mice (Biomedical Sciences Research

Center "Alexander Fleming", Vari, Greece), sex, weight (19–25 g), and age (8–10 week) matched, were anaesthetized with intraperitoneal ketamine/xylazine injection. After minimal skin, fascia, and muscle dissection over the left lateral chest wall, mice received intrapleural injections of 500 μL PBS-BSA 1% (325 mOsm/kg H_2O), followed by wound closure using continuous ethilon suture. Mice received intraperitoneally dexamethasone at 1 mg/kg body weight or PBS (control), 5 min after the induction of hydrothorax. All animals were observed until full recovery without showing any sign of distress. Dexamethasone and BSA were purchased from Sigma-Aldrich Chemie, Munich, Germany.

Experimental groups

Mice were distributed in four groups (six animals per group). Two groups served as control and two groups were treated with dexamethasone. Control and treated animals were sacrificed 2 and 4 h after the induction of hydrothorax. The pleural cavity was opened and the pleural fluid was completely aspired bilaterally via a 1-mL syringe and a 26-gauge needle and its volume was measured using a micropipette.

In order to validate the reliability of the method of fluid aspiration, five additional mice were intrapleurally injected with 500 μL PBS-BSA 1% containing 4 mg/mL Evans' blue dye and they were sacrificed 5 min after the injection. The mean ± SEM volume of the aspired fluid was found to be 497.4 ± 1.7 μL and the corresponding isosmotic fluid clearance was calculated to be 0.52 ± 0.34 μL/min. It should also be noticed the above studies confirmed the accuracy of the intrapleural injection technique since negligible leak of the dye in the chest wall was observed only in two animals. This group of animals is cited in our figure as the "retention volume" group.

Statistical analysis

All data are normally distributed and expressed as mean ± standard error of mean (SEM). Comparison of the differences of the mean values between untreated and dexamethasone-treated groups was calculated using unpaired Student's t test. Differences among multiple groups were compared using one-way ANOVA. Post hoc analysis was performed with the Bonferroni test. Values of $p < 0.05$ were regarded as significant. Statistical analysis was performed with GraphPad Prism v4.0c for Mac OS X.

Results

Five minutes after induction of hydrothorax in the group of mice ($n = 5$) used for validation of the aspiration method (retention volume group) were sacrificed and the mean pleural fluid volume was found to be 497.4 ± 1.7 μL which was not significantly different from the initial volume of 500 μL that was injected.

Two hours after the induction of hydrothorax, the mean pleural fluid volume of the dexamethasone treated mice was significantly lower compared to that of untreated mice (213.3 ± 23.19 vs 368.3 ± 23.86 μL, respectively; $p = 0.0009$; Fig. 1a). Similarly, 4 h after the induction of hydrothorax, the mean pleural fluid volume of the dexamethasone-treated mice was significantly lower compared to that of untreated mice (155 ± 18.93 vs 316.66 ± 42.16 μL, respectively; $p = 0.0057$; Fig. 1b). No differences were observed between the mean pleural fluid volume of the 2-h control group and the 4-h control group ($p = 0.31$) or between the mean pleural fluid volume of the 2-h dexamethasone group and the 4-h dexamethasone group ($p = 0.08$).

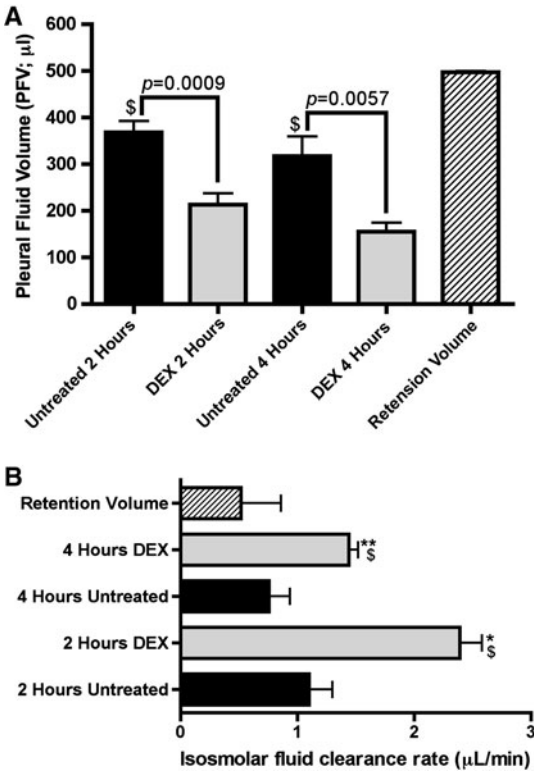

Fig. 1 **a** Effect of dexamethasone on pleural fluid volume after induction of hydrothorax. The remaining pleural fluid volume (μL) of the retention volume group and the untreated and dexamethasone-treated group of mice 2 and 4 h after the induction of isosmotic hydrothoraces of 500 μL. **b** Effect of dexamethasone on pleural fluid absorption rate after induction of hydrothorax. The pleural fluid absorption rate (μL/min) of the untreated and dexamethasone treated group of mice 2 and 4 h after the induction of isosmotic hydrothoraces of 500 μL. Values are mean ± SEM. *Bars* $^{*}p < 0.05$ vs 2 h untreated group, $^{**}p < 0.05$ vs 4 h untreated group, $^{\$}p < 0.05$ vs 2 and 4 h untreated groups

We then compared the rate of pleural fluid absorption between different groups during the "0- to 2-h" period and during the "2- to 4-h" period. Dexamethasone-treated animals were characterized by significantly higher rates of absorption both during the initial (2.39 ± 0.19 vs 1.10 ± 0.2 μL/min, for treated and control animals, respectively; $p = 0.0009$; Fig. 1b), and during "2- to 4-h" period (1.44 ± 0.08 vs 0.76 ± 0.18 μL/min, for treated and control animals, respectively; $p = 0.0062$; Fig. 1b). No significant difference was found when the rate in the untreated groups between the two periods of time were compared ($p = 0.235$).

Discussion

In the present study, we investigated the effect of corticosteroids in pleural fluid clearance after the induction of isosmotic hydrothoraces in mice. We demonstrated that a single-dose dexamethasone enhanced the rate of pleural fluid clearance resulting in decreased residual pleural fluid volume both at 2 and 4 h after the creation of hydrothoraces. More specifically, after administration of dexamethasone, we showed that its effect is 117% at the 2-h period and 89% at the 4-h period enhanced compared to the base-line rate.

Corticosteroids are frequently used to treat patients with pleural effusions of certain etiologies such as autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis), post-cardiac injury syndrome, eosinophilic pleural effusions [1–3]. In addition, though not being part of the standard anti-tuberculous treatment, a recent meta-analysis suggested that corticosteroid treatment accelerated the resolution of tuberculous pleural effusions [4]. This effect is thought to be secondary to their anti-inflammatory properties, though there are no studies regarding the underlying mechanism. Based on the findings of the present study, we assume that the pleural fluid limiting effect of dexamethasone may be partly associated with the pleural fluid absorption rate accelerating activity of the drug.

Even though the impact of corticosteroids in pleural fluid absorption is clearly demonstrated in the present study, the underlying pharmacological actions remain elusive. To address this issue, it is important to consider the different mechanisms regulating pleural fluid clearance: (1) absorption from visceral pleura capillaries due to Starling forces; (2) lymphatic drainage through the stomata located on the parietal pleura; (3) transcytosis of proteins through parietal and visceral pleural mesothelial cells [12, 13]; and (4) transcellular ion and water transport through parietal and visceral pleura mesothelial cells (through channels like ENaC, Na^+-K^+ ATPase, Aquaporin-1, etc.) [11, 13–15]. In the present studies, we created isosmotic hydrothoraces of the same volume in both dexamethasone-treated and control animals, thus the osmotic and hydrostatic pressures (involved in the first mechanism) are the same. An effect of corticosteroids in lymph vessel contraction has not been described. Aquaporin-1 expression on pleural membranes could be up-regulated by corticosteroids [14]. However, its involvement in the effects observed in our studies should be ruled out since aquaporin-1 does not contribute to isosmotic hydrothoraces absorption [14, 15]. Finally, no data exist on the effect of corticosteroids in trancytosis.

On the other hand, corticosteroids have been previously shown to activate the ion transporting systems (i.e. ENaC, Na^+–K^+ ATPase, Na^+/H^+ exchanger) in epithelial cells [7–11]. These proteins are thought to participate in pleural fluid removal from the pleural space [12, 13]. Moreover, it has been recently reported that dexamethasone rapidly increases ion transport on sheep visceral and parietal pleura, as well as on sheep visceral peritoneum [16, 17]. In both cases, the quoted effect was attributed to up-regulation of ion transporting channels on the mesothelial plasma membrane of pleural cells. The above may provide an explanation of our findings regarding the acceleration of the pleural fluid absorption in our system, which may be related with corticosteroid-induced up-regulation of ion transport [7–11] in mesothelial cells.

In conclusion, we herein demonstrate that corticosteroid treatment accelerates pleural fluid clearance in a mouse model of isosmotic hydrothoraces. This newly reported property of dexamethasone may partly account for the clinical observation of faster resolution of pleural effusions when corticosteroids are administered in patients with pleural effusions of certain etiologies.

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