Dexamethasone decreases the transmesothelial electrical resistance of the parietal and visceral pleura

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Abstract The effect of dexamethasone on the transmesothelial electrical resistance ($R_{TM}$) of sheep pleura was investigated by Ussing chamber experiments. Our results show that dexamethasone decreases the $R_{TM}$ of sheep pleurae, in part by stimulation of glucocorticoid receptors. This finding may be of importance in regard to the faster resolution of corticosteroid-treated pleural effusions.

Keywords Corticosteroids · Pleural effusion · Ussing system

Introduction Pleural effusion is the accumulation of excess pleural fluid in the pleural cavity. In a number of diseases, such as tuberculosis, postcardiac injury syndrome, systemic lupus erythematosus, rheumatoid arthritis and sarcoidosis, patients often develop pleural effusions. In these cases, administration of corticosteroids results in acceleration of the effusion resolution through a mechanism that is generally attributed to the anti-inflammatory effects of steroids [1]. In physiological conditions solute coupled liquid absorption through the mesothelium is the main pathway of pleural fluid absorption, while in pleural effusions lymphatic drainage is the main route of fluid absorption [2–4].

Glucocorticoid receptors have been identified by immunostaining in mesothelial cells of parietal and visceral peritoneum in rats [5]. Dexamethasone is a widely used synthetic glucocorticoid due to its anti-inflammatory action that exhibits both genomic and non-genomic effects on cells and tissues [6]. Studies of dexamethasone in animal models have shown that it reduces the total cell and eosinophils count in pleural lavage of pneumothorax-associated pleural eosinophilia in mice [7].

The direct effect of dexamethasone on the pleural membrane has never been studied. However, a recent study on visceral peritoneal membrane has shown that dexamethasone results in an increase of the ionic permeability of the visceral peritoneum, as has been demonstrated by transmesothelial electrical resistance measurements [8]. Furthermore, sex-steroids, such as estradiol and progesterone, have been found to alter the electrical properties of stripped specimens of sheep pleura, suggesting implications in the resolution of effusions that are accompanied with elevated levels of these hormones, probably due to downregulation of mesothelial solute coupled liquid absorption [9].

The objective of this study was to investigate the effect of dexamethasone on the pleural transmesothelial electrical resistance ($R_{TM}$) and thus on the ionic permeability of sheep visceral and parietal pleura.
Materials and methods

Specimen collection and preparation

Intact sheets of visceral and parietal pleura were obtained from 72 adult sheep (males and females). The samples were collected from the slaughterhouse immediately after the death of the animal (time of warm ischemia close to 0) and transferred to the laboratory in oxygenated Dulbecco Modified Eagle’s Medium (DMEM) buffer at 4°C within 30 min of the death of the animal. The pieces of visceral pleura were carefully stripped from the underlying lung, while the parietal ones were carefully stripped from the chest wall and then examined for evidence of holes or adherent tissue by visual inspection. Care was taken to touch the surface as little as possible. Pieces of parietal pleura not likely to contain stomas were used, as suggested from anatomical studies in sheep [10, 11].

Transmesothelial electrical resistance measurements

Visceral or parietal pleura specimens were carefully mounted in Ussing chambers (Dipl.-Ing. K. Mussler Scientific Instruments, Aachen, Germany) with an opening surface area of 1 cm². Tissues were bathed with 4 ml of Krebs–Ringer bicarbonate (KRB) solution on each side of the membrane, continuously oxygenated with 95% O₂/5% CO₂ circulated by gas lift. The KRB solution was balanced at pH 7.4 and contained (in mM) 117.5 NaCl, 1.15 NaH₂PO₄, 24.99 NaHCO₃, 5.65 KCl, 1.18 MgSO₄, 2.52 CaCl₂, and 5.55 glucose.

Two pairs of Ag/AgCl electrodes monitored the transmesothelial electrical resistance (Rₜₘ) in ohms per square centimeter (Ω cm⁻²) under open-circuit conditions. The Rₜₘ was measured every 60 s. Experiments were conducted simultaneously in six computer-controlled chambers (Clamp version 2.14 software: AC Micro-Clamp, Aachen, Germany). Transmesothelial electrical resistance was measured in the basal state (that is, at the end of an equilibration time of 10–40 min) and after the addition of different substances. Because active transport of ions is influenced by temperature, the Ussing chambers were held at 37°C.

The voltage responses to applied current pulses of given amplitude (50 μA) and duration (200 ms) were measured. The Rₜₘ was calculated by automatically deducing the initially measured resistance of the solution. Changes in Rₜₘ after the addition of the chemicals were determined as percent (%) changes (ΔRₜₘ).

Experimental procedure

The mesothelial cell membrane side that in vivo faces the pleural fluid is referred to as apical, and the one that in vivo faces the blood supply is referred to as basolateral.

Measurements of Rₜₘ were made before and after exposure to substances for given time points (at minutes 1, 3, 5, 10, 15, 20 and 30). Control transmesothelial electrical resistance (Rₜₘ)—tissue with KRB solution—measurement performed across the parietal pleura was (n = 6) and across the visceral pleura (n = 6). The control resistance remained unchanged during the period of 30 min, which was the duration of the experiments in all cases. Additionally, another set of control experiments was performed where 4 μl of ethanol, which served as the vehicle of dexamethasone and mifepristone solutions, was added in both parietal (n = 6) and visceral (n = 6) pleura. Similarly no changes in the Rₜₘ were observed within 30 min.

In the initial set of experiments, dexamethasone (final concentration of 10⁻⁵ M) was added apically on the parietal (n = 6) and visceral (n = 6) pleura, as well as basolaterally on the parietal (n = 6) and visceral (n = 6) pleura, in different pleura specimens.

In another set of experiments the glucocorticoid receptor antagonist mifepristone (final concentration of 10⁻⁵ M) and mifepristone (10⁻⁵ M) plus dexamethasone (10⁻⁶ M) was added apically on the parietal (n = 6) and visceral (n = 6) pleura, as well as basolaterally on the parietal visceral (n = 6) and visceral (n = 6) pleura.

All solutions were freshly prepared before each experiment, heated to 37°C, and bubbled continuously with a 95% O₂/5% CO₂ gas mixture. All chemicals were purchased from Sigma-Aldrich Chemie GmbH, Munich, Germany.

Statistical analysis

Statistical analyses were performed with GraphPad Prism v4 for Mac OSX (GraphPad Software Inc., San Diego, CA). All data are expressed as mean ± SEM. The results presented in this study are the means of six different experiments in each case. The probability of error for comparison of the mean values was calculated using one-way ANOVA or two-way ANOVA with Bonferroni post-test where appropriate. Values of P < 0.05 were regarded as significant.

Results

The control transmesothelial electrical resistance (Rₜₘ)—tissue with KRB solution—across the parietal pleura was 19.83 ± 1.28 Ω cm⁻² (n = 6), and across the visceral pleura 18.67 ± 0.84 Ω cm⁻² (n = 6).

In Fig. 1, a sample experiment of addition of dexamethasone on parietal pleura apically is shown. Addition of an equal volume of vehicle of dexamethasone and mifepristone (ethanol) alone in both parietal (n = 6) and
Addition of dexamethasone (10⁻⁶ M) apically on the parietal pleura resulted in a gradual decrease of the $R_{TM}$, being statistically significant after 10 min ($\Delta R_{TM} = -30.33 \pm 4.1\%; \ P < 0.05$), which was maintained for the whole experimental procedure (Fig. 2a). Addition of dexamethasone (10⁻⁶ M) basolaterally on the parietal pleura resulted in a gradual decrease of the $R_{TM}$, being statistically significant after 5 min ($\Delta R_{TM} = -24.84 \pm 5.83\%; \ P < 0.05$), which was maintained for the whole experimental procedure (Fig. 2b).

Similarly, when dexamethasone was added on the visceral pleura apically, it induced a gradual decrease that was statistically significant after 10 min ($\Delta R_{TM} = -22.5 \pm 2.29\%; \ P < 0.05$) and was maintained till the end of the experiment (Fig. 2c). Basolateral addition of dexamethasone had a similar effect (Fig. 2d), being significant after 5 min ($\Delta R_{TM} = -16.16 \pm 4.19\%; \ P < 0.05$).

Addition of the glucocorticoid receptor antagonist mifepristone, apically and basolaterally, had no effect either

Fig. 1 Sample experiment showing a decrease in $R_{TM}$ after addition of dexamethasone on the parietal pleura apically, throughout the experiment

Fig. 2 Effect of dexamethasone (10⁻⁶ M; DEX), mifepristone (10⁻⁵ M; MIF) and combined effect of mifepristone (10⁻⁵ M) plus dexamethasone (10⁻⁶ M) on changes in $R_{TM}$ expressed as the % difference from the baseline measurement ($\% \Delta R_{TM}$) to the apical (a) and basolateral (b) on the parietal pleura. Effect of dexamethasone (10⁻⁶ M; DEX), mifepristone (10⁻⁵ M; MIF) and combined effect of mifepristone (10⁻⁵ M) plus dexamethasone (10⁻⁶ M) on changes in $R_{TM}$ expressed as the % difference from the baseline measurement ($\% \Delta R_{TM}$) to the apical (c) and basolateral (d) on the visceral pleura. Values are means ± SEM, n = 6 experiments in each case. *P < 0.05 versus control, #P < 0.05 versus dexamethasone.
on parietal or on visceral pleura (Fig. 2). However, on the parietal pleura, administration of mifepristone apically delayed and reduced the effect of dexamethasone 20 min after addition (−22.66 ± 5.35% vs. −51.66 ± 3.74%; P < 0.01; Fig. 2a) and 5 min after addition basolaterally (−6.5 ± 3.99% vs. −24.83 ± 5.82%; P < 0.05; Fig. 2b). Contrary to that, mifepristone did not alter the effect of dexamethasone when it was placed apically on the visceral pleura (Fig. 2c), while basolaterally the effect of dexamethasone was delayed and reduced, being significant 15 min after addition (−13.16 ± 10.26% vs. −37.16 ± 2.39%; P < 0.05; Fig. 2d).

Discussion

The results of the present study clearly show that dexamethasone has a potent and rapid effect on the parietal and visceral pleura. Dexamethasone induces a significant decrease on the transmesothelial resistance within 5–15 min, depending on the side where it has been added. The specific glucocorticoid antagonist mifepristone reduces and delays this effect on both the parietal and visceral pleura. The above findings suggest that (1) the effect of dexamethasone is rapid and (2) in part mediated through stimulation of glucocorticoid receptors.

A number of in vivo, in vitro and molecular studies have highlighted the role of mesothelial ionic transporters in pleural fluid turnover [2–4, 9, 12–16, 20]. In all in vitro studies regarding pleura electrophysiology, electrical resistance was found to be low [9, 12–16, 20]. The early proofs of the importance of mesothelial ionic transporters are derived from in vivo experiments in rabbits where inhibitors of these transporters reduced pleural fluid absorption rates [2]. Later in vitro studies using the same inhibitors had similar results as interpreted by the increase in the transmesothelial resistance that was observed [3, 12–16]. The above suggest that although the electrical resistance of the pleura is much lower than other epithelia, it is an important regulator of solute coupled liquid absorption, which is one of the main mechanisms of pleural fluid turnover.

The R_{TM} assessed in our study is an established surrogate of mesothelial permeability and is inversely correlated to ionic permeability [12–20]. Our results on the effect of dexamethasone in decreasing the R_{TM} and thus enhancing the ionic transport on the pleural mesothelium are in accordance with previous studies. A decrease in transepithelial resistance following corticosteroid treatment on the apical membrane of porcine and bovine retinal pigment epithelium has been reported [21].

Our findings are in accordance with previously reported findings in another serosal membrane, the visceral peritoneum [8]. In the study of Karioti and co-workers dexamethasone has been found to induce similar effects on the peritoneum, and this was an effect also mediated in part through stimulation of glucocorticoid receptors. Steroids exhibit non-genomic effects, such as stimulation of ionic transport when acting on epithelial cells [6]. This is achieved by translocation of ion transporting proteins like epithelial sodium channel (ENaC) and sodium–potassium pump (Na⁺–K⁺-ATPase) on the plasma membrane of epithelial cells as well as by up-regulation of their activity [22–24]. However, translocation of these transporters is known to occur at a later time period than the 5-10 min in which we observed the decrease in R_{TM} in our study. Therefore, an up-regulation in ENaC and Na⁺–K⁺-ATPase activity may be the most probable underlying mechanism. Potential passive flux of fluid and ions occurring due to the effect of dexamethasone on resistance is a possibility that we cannot rule out.

Additionally, dexamethasone has been reported to restore the integrity of the epithelial barrier when damaged by inflammatory agents, such as transforming growth factor beta (TGFβ) and tumor necrosis factor alpha (TNFα), through reorganization of the apical tight junctions, reducing thus paracellular ion transport [25, 26]. In this context, any effect of dexamethasone on tight junctions in the present experiment would result in exactly the opposite results, i.e., an increase of the R_{TM} and thus a decrease of permeability. Therefore, any actions of dexamethasone on the tight junctions could not explain our results. Moreover, in our experimental procedure, specimens of healthy pleura without signs of inflammation isolated from healthy animals were used, rendering any important action of dexamethasone and tight junctions less likely.

Contrary to that, in corticosteroid-treated pleural effusions from various etiologies where inflammatory agents are present [1], both quoted properties of dexamethasone, the upregulation of solute coupled liquid absorption along with the restoration of the mesothelial barrier integrity, may provide an explanation to the acceleration of pleural effusion resolution following corticosteroid treatment.

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