Work in progress report - Experimental

Human parietal pleura present electrophysiology variations according to location in pleural cavity

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Abstract

The aim of the study was to investigate if human pleura from different anatomical locations presents electrophysiology differences. Specimens were stripped over the 2nd–5th rib (cranial), 8th–10th rib (caudal), and mediastinum during open surgery and were mounted between Ussing chambers. Amiloride and ouabain were added towards mesothelial surface and trans-mesothelial potential difference (PD_{tm}) was measured after 1, 5, 10 and 20 min. Trans-membrane resistance (R_{tm}) was calculated from Ohm’s law. R_{tm} increased after amiloride addition, for cranial (net increase of 0.40 Ω·cm²) and caudal (1.16 Ω·cm²) pleural pieces. Mediastinal pleura R_{tm} remained unchanged (0.09 Ω·cm²). R_{tm} increase was higher for caudal than cranial (P=0.029) or mediastinal tissues (P=0.002). R_{tm} increased after ouabain addition for cranial (1.35 Ω·cm²) and cranial (0.56 Ω·cm²) pleural pieces. Mediastinal pleural tissue did not respond (0.20 Ω·cm²). Caudally located pleura responded greater than cranial (P=0.043) or mediastinal (P=0.003) pleural tissues. Human pleura shows electrophysiology differences according to the location within the pleural cavity. Surgeons may waste mediastinal pleura when needed but should leave intact caudal parietal pleura, which seems to be electrophysiologically the most important part of the pleural cavity.

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1. Introduction

The importance of pleural stripping during thoracic surgery is pinpointed in numerous and various pathology. Parietal pleura is ‘stripped off’ from upper thoracic cage during pleurodesis due to pneumothorax, while thickened parietal pleura is stripped off in an effort to optimize thoracic cage movement after decortication surgery for thoracic empyema [1]. Mediastinal pleura is wasted during oncology surgery where lymph node picking of the mediastinum is performed.

From a physiology aspect, pleural mesothelium seems important for pleural fluid recycling via active electrolyte transportation which is shown to include different ion transporters moving electrolytes and water in and out from the pleural cavity [2–6]. However, pleural and pleural cavity physiology seems to be dissimilar from region to region [7], as for example pleural fluid becomes thicker from apex to diaphragm [8], while lymphatic stomata appear to be more numerous in the diaphragmatic pleura [9, 10], but absent on visceral and apical pleura of some species [11, 12].

The aim of the study is to identify differences of the electrophysiology of the healthy human pleura originated from different locations of the pleural cavity, in order to indicate, from a physiology aspect, the most important region which should be brought to the surgeons’ attention during surgery.

2. Material and methods

Thirty-nine specimens from three different pleural locations were stripped off from patients who underwent pulmonary parenchyma resection for lung cancer via thoracotomy for diagnostic and/or therapeutic purposes. The resected lung mass was not in proximity to the stripped specimens of the pleural piece used for the study. Patients who developed pleural effusion prior to surgery were excluded from the study. A piece of each stripped specimen was sent for histopathology examination. The remaining specimen was placed in Krebs (KRB) solution pre-oxygenated at 95% O₂–5% CO₂ and was transferred to the laboratory within 30 min (using a carrier fridge ensuring that the tissue will be carried at 4 °C). All specimens included in the study were proven to be free of any disease by the histopathology report. In order to obtain anatomically different specimens, pleural sheets were stripped off from the chest wall, two to three ribs above (2nd–5th, cranial) or below the thoracotomy incision (7th–10th, caudal), as well as from the mediastinum above the pulmonary hilum.
bilaterally. Mediastinal pleural tissues were easily obtained as it is free standing and no stripping is required [8].

The study was approved by the Local Ethics Committee and signed consent was obtained from all the participants in the study patients.

The pleural pieces were examined thoroughly for holes, fat tissue, or residual blood clots after stripping. The KRB solution mentioned above and throughout the whole study, 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. The KRB was bubbled with 95% O₂–5% CO₂.

The pleural tissues were mounted between two ‘Ussing-type chambers’ connected to glass reservoirs. Silicone was applied along the rim of each O-ring of the chambers, to ensure tightness, as well as to minimize edge effect [5]. Each chamber had a total volume (including the reservoir) of 20 ml. The cross-sectional area of the exposed tissue between the reservoirs was 1.43 cm². Since active transport of ions is influenced by temperature, all measurements of electrical potential difference were made at 37 °C. The tissue was bathed with KRB solution on both sides, and bubbled continuously with a 95% O₂–5% CO₂ gas mixture, in order to ensure tissue viability.

The trans-mesothelial potential difference (PDₘₐ) across the tissue was measured with 3 M KCl 3% agar bridges placed 3 mm on either side of the membrane. These bridges were connected on either side to Ag/AgCl electrodes, and the output was amplified (model DVC-3 with input impedance 10¹² Ω, World Precision Instruments, USA). In order to determine the voltage response to an external current, direct current provided by a voltage-clamp apparatus (model DVC-1000, World Precision Instruments, Sarasota, Florida, USA) was applied on the tissue via 3 M KCl agar bridges placed in the reservoir connected to each hemichamber.

The surface of the pleura that faces in vivo the pleura cavity, will be referred to as the ‘mesothelial surface’ and the surface that faces the chest wall, will be referred to as the ‘interstitial surface’.

The pleural tissue was equilibrated for 30 min and Trans-mesothelial potential difference (PDₘₐ) was measured for 20 min in the absence as well as after current application of variable intensity (0–±400 μA) [5]. The PDₘₐ measured at 20 min after the tissue equilibration, consists of the ‘control potential difference’. The number of control experiments was thirty-nine.

Na⁺-channel blocker amiloride 10⁻⁵ M (Sigma Chemical Co., USA) and Na⁺-K⁺-pump inhibitor ouabain 10⁻³ M (Sigma Chemical Co., USA) solutions were added in a ‘bolus’ manner. In order to ensure that results recorded are due to drug action and not to mechanical perturbation, experiments were conducted by using only KRB solution (data not shown as no changes of PDₘₐ were observed). PDₘₐ after electrical stimulation with current application (range 0–±400 μA) was measured 1, 5, 10 and 20 min after each solution addition. All solutions were freshly prepared before each experiment, heated to 37 °C and continuously bubbled with 95% O₂–5% CO₂ gas mixture.

Trans-mesothelial resistance (Rₘₐ) was calculated from PDₘₐ according to Ohm’s law [6]. Control Trans-mesothelial Resistance was calculated from control PDₘₐ measured after equilibration of the tissue. The mean net increase of Rₘₐ within the 1st minute was calculated by subtracting the mean Rₘₐ from the mean control values for each pair of measurements.

Statistical analysis was performed using the SPSS, version 10.0 for Windows, installed in the University of Thessaly. Data are expressed as mean Rₘₐ±standard error of mean (S.E.) or as the mean net increase of Rₘₐ above control level at the 1st minute. Statistical significance between different anatomical regions was determined by ANOVA–Bonferroni post hoc analysis for multiple comparisons. P-values <0.05 were accepted as significant.

3. Results

The mean control measurements of the three anatomical region groups were calculated to be statistically different (ANOVA, P=0.021, df=39). Control Rₘₐ of cranial parietal pleura (20.50±0.5 Ω·cm²) was statistically different (P=0.029) from caudal parietal pleura control Rₘₐ (18.44±0.4 Ω·cm², Fig. 1). Mediastinal pleural control Rₘₐ was similar to the cranial pleural control Rₘₐ (20.57±0.6 Ω·cm²) and similarly higher from the caudal control Rₘₐ (P=0.018, Fig. 1).

The mean net Rₘₐ increase within the 1st minute after amiloride addition towards the mesothelial surface (Fig. 2) between the three anatomical region groups (cranial, caudal, mediastinal) was calculated to be statistically different (ANOVA, P=0.001, df=21). Mean net increase of Rₘₐ for caudal and cranial pleural regions was 1.16±0.4 Ω·cm² and 0.40±0.4 Ω·cm², respectively. The difference was statistically significant (P=0.029). The mean net Rₘₐ increase of

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Fig. 1. Control trans-mesothelial resistance Rₘₐ of human pleura originated from cranial parietal, caudal parietal and mediastinal pleura. Values are presented as mean trans-mesothelial resistance (Rₘₐ) in Ω·cm²±standard error of mean (S.E.) of 39 experiments. *P<0.05 vs. caudal pleura control Rₘₐ.

4. Discussion

The main finding of this study is that the caudal pleural regions demonstrated more intense electrical changes than the cranial or mediastinal regions after each drug (amiloride or ouabain) addition. The mediastinal pleura demonstrated a very weak electrical response too. These observations indicate the existence of anatomical variations of the electrophysiological properties between different regions of the same pleural petal (parietal).

Results from control studies experiments showed that the caudal parietal pleural tissue had lower $P_{TM}$ and $R_{TM}$ after equilibration and stimulation only by current, in the absence of ion transporter blockers, and thus it was more ‘leaky’ than the other parts of the pleural cavity tissues and thus more permeable to electrolytes and water [5].

The stimulation of the pleural specimens by current in the presence of ion transporter blockers (amiloride and ouabain) revealed a greater response of the caudal parietal pleural tissues. Caudal regions reacted significantly greater than the cranial and mediastinal regions after amiloride addition, which suggests that the lower pleural cavity regions might be equipped with more Na$^+$ channels, where additionally more lymphatic ‘ stomata’ are present [7]. Caudal regions also reacted greater than the cranial and mediastinal regions after ouabain addition, suggesting that caudal regions of pleural membrane are equipped with more Na$^+$–K$^+$ pumps.

Comparing the three different parts of human pleura (cranial parietal, caudal parietal and mediastinal) it is concluded that the human caudally located parietal pleura is the most permeable (control studies) and most active electrically (studies with ion transporter blockers) and consequently, if the theory of active trans-mesothelial permeability is valid [2], then probably interferes the most in the overall exchange of small solutes and liquid. The alteration of the electrical activity of the mediastinal pleura as a response to ion transporter blockers was present but negligible, indicating that cellular transportation is possible to occur across this tissue to a limited extent, but most probably this electrical activity cannot provide a countable force to the overall pleural permeability within the pleural cavity [13]. Such an assumption if proven to be valid will constitute mediastinal pleura as a simple supportive structure rather than an active barrier, as is the case for the other parts of the pleural cavity. Experimental results from the present study also suggest that the electrophysiological properties of human parietal pleura follow a similar cranial–caudal axis, as is the case for pleural pressure and pleural liquid thickness [7–9].

The knowledge provided by this study could change the overall consideration of surgical manipulations concerning parietal and mediastinal pleura. Thoracic surgeons can safely resect parietal pleura of the apex and mediastinal pleura to achieve pleurodesis during surgery for pneumothorax or to create pleural flaps for any use during surgery. Moreover, knowledge of this study may be of importance for the surgical treatment of empyema thoracis. The goal of surgery for empyema thoracis in the organizing stage is to ‘strip off’ the inelastic peel or cortex, which covers the visceral pleura and allows full expansion of the lung [14].
Visceral pleura, which remain thin under the fibrous peel, should be left intact in order to avoid air leaks [1, 14]. Parietal pleura is thickened and many authors have suggested its stripping during empyema surgery to achieve normal thoracic cage movement. Stripping of the thickened parietal pleura may lead to important blood loss, while in physiologic studies in patients following decortication it was found that the degree of improvement was similar whether or not parietal pleural decortication was performed [1, 15]. This study enhances the idea of leaving as intact as possible the parietal pleura that covers the lower parts of the pleural cavity, which is almost always involved in the empyema process, because the loss of its important electrochemical function can surcharge negatively to postoperative rehabilitation and pleural effusion mobilization, apart from the side-effects described before.

In conclusion, human pleura shows electrophysiology differences according to the location within the pleural cavity. From electrophysiology aspect, parietal pleura seems to be active while mediastinal pleura appears to be a simple separating or connective structure. Parietal pleura located over the lower parts of the pleural cavity seems to be more active than parietal pleura located over the upper parts, suggesting that the lower pleural regions can be more permeable, knowledge which may be an important consideration when performing thoracic surgery.

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References