Low glucose level and low pH alter the electrochemical function of human parietal pleura

V.K. Kouritas^{*,#}, C. Hatzoglou^{*}, C.N. Foroulis[#], A. Hevas[#],
K.I. Gourgoulidis[†] and P.A. Molyvdas^{*}

ABSTRACT: The aim of the present study was to investigate whether low glucose and pH level, which are usually measured in complicated pleural effusions, alter the electrochemical function of healthy human parietal pleura.

Parietal pleural pieces were stripped from 66 patients during thoracic surgery and were mounted in Ussing chambers. Krebs' solutions containing different glucose levels (0, 40 and 100 mg) and balanced at different pH levels (7.4, 7.3 and 7.2) were added to the pleural cavity surface of the pieces. Transmesothelial potential difference was measured at various time-points as an electrophysiological variable and transmesothelial resistance (R_{TM}) was calculated using Ohm's law.

When normal-glucose Krebs at pH 7.45 was used, R_{TM} remained unchanged over time, but when low-glucose Krebs was used, R_{TM} decreased. Krebs without glucose caused the greatest decrease in R_{TM} . Use of low-pH Krebs decreased R_{TM} . The lower the pH of the Krebs, the faster the decrease in R_{TM} and the greater the effect. The decrease in R_{TM} was greater with low-pH than with low-glucose Krebs. Low glucose and low pH caused an additive decrease in R_{TM} .

Low glucose concentration and low pH cause alteration of the electrochemical function of human parietal pleura and could act as agents that lead to further exudate progression.

KEYWORDS: Exudates, glucose, human, parietal pleura, pH, transmesothelial resistance

Low glucose level and low pH often characterise effusions complicating malignancies, mainly primary lung cancer, or autoimmune diseases such as rheumatoid arthritis. They are considered as biochemical indicators of complicated parapneumonic effusions [1–3], which can aid diagnosis [1, 3].

Possible explanations for the decreased glucose level or low pH of these effusions include: increased utilisation of glucose by abnormal (e.g. bacterial metabolism) or defence cells (e.g. leukocytes) in the pleural cavity; the abnormal transference of substrates across the diseased or inflammatory pleural membrane [1, 3–5]; and the phagocytosis of bacteria along with the release of cell wall components [3, 4, 6, 7].

The purpose of the present study was to investigate whether low glucose level and low pH can alter the electrochemical function of the healthy human parietal pleura.

MATERIALS AND METHODS

Study subjects

Human parietal pleura specimens were obtained from 66 patients who underwent thoracic surgery. The mean age was 62.5 yrs. The mean serum glucose was 108 mg·dL⁻¹. Patients who developed parapneumonic effusions or whose pre-operative serum glucose level was abnormal, were excluded from the study. A piece of each stripped pleural specimen was sent for histopathological examination. All specimens included in the study were shown by the histopathological report to be free of disease. The remainder of each specimen was placed in Krebs' (KRB) solution, pre-oxygenated with 95% O₂:5% CO₂, and transferred to the laboratory within 30 min.

The study was approved by the local ethics committee (University of Thessaly, Larissa, Greece) and informed consent was obtained from all patients participating in the study.

AFFILIATIONS

^{*}Dept of Physiology, University of Thessaly Medical School, Depts of [#]Cardiothoracic Surgery, and

[†]Thoracic Diseases, Larissa University Hospital, Larissa, Greece.

CORRESPONDENCE

V.K. Kouritas

Dept of Physiology
University of Thessaly Medical School

22 Papakiri St
412 22

Larissa

Greece

Fax: 30 2410670100

E-mail: kouritas@otenet.gr

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STATEMENT OF INTEREST

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Study design

Complicated pleural effusions were simulated with KRB solutions containing different glucose concentrations, balanced at different pH levels, which were added to the pleural surface of the tissue. Electrophysiological parameters were measured.

Methods

The specimens were examined for holes, fat tissue or residual blood clots. The initial KRB was balanced at pH 7.45, cooled to 4° C, 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 specimens were mounted as planar sheets between Ussing-type chambers. The tissue was bathed with KRB on both sides and bubbled with a 95% O₂:5% CO₂ gas mixture heated to 37°C.

The transmesothelial potential difference (PDTM) was measured with 3 M KCl 3% agar bridges connected to silver/silver chloride electrodes, and the output was amplified (model DVC-3; World Precision Instruments, Sarasota, FL, USA). Current was provided by a voltage-clamp apparatus (model DVC-1000; World Precision Instruments).

The specimens were equilibrated for 30 min. Control PDTM was measured in the absence of current and after the application of currents ranging -400–400 µA [8]. A total of 66 control experiments were carried out.

KRB solutions containing 100, 40 and 0 mg glucose (n=10 experiments for each glucose concentration) were added to the pleural surface. KRB solutions balanced at pH 7.4, 7.3 and 7.2 (n=9 experiments for each pH) were also used. Experiments were also conducted with KRB solutions without glucose, balanced at pH 7.2 (n=9 experiments). The chamber facing the outer-pleural surface was filled with 5.55 mM glucose KRB balanced at pH 7.45, ensuring tissue viability [9, 10]. PDTM was measured 1, 5, 10, 30, 45 and 60 min after the addition of each solution.

Transmesothelial resistance (RTM) was calculated from PDTM using Ohm's law [8]. Change in RTM was calculated by subtracting the mean RTM from the mean control values at the same time-point.

Analysis

Data are expressed as mean±SEM or as the change of RTM. Statistical significance was determined by paired t-test. The statistical significance of RTM changes between groups was determined by ANOVA. A p-value <0.05 was accepted as significant.

RESULTS

RTM remained constant throughout the experiments using normal-glucose KRB at pH 7.45.

Low-glucose KRB

RTM decreased 10 min after the addition of 40 mg glucose KRB (fig. 1a) and decreased significantly after 45 (p=0.020) and 60 min (18.65±0.3 Ω·cm⁻²; p=0.009). RTM was similarly decreased 5 min after the addition of 0 mg glucose KRB (fig. 1a), and was significantly decreased from 10 min (p=0.034) until 60 min (18.20±0.3 Ω·cm⁻²; p=0.001). The net

RTM change was more intense (p=0.024) 30 min after the addition of glucose KRB than 30 min after the addition of 40 mg glucose KRB (fig. 1b). This was also true after 45 (p=0.011) and 60 min (0.67±0.2 Ω·cm⁻² for 40 mg glucose KRB versus 1.12±0.2 Ω·cm⁻² for 0 mg glucose KRB; p=0.009).

Low-pH KRB

RTM had decreased 60 min (18.44±0.2 Ω·cm⁻²; p=0.049) after the addition of pH 7.4 KRB (fig. 2a). RTM had decreased 10 min after the addition of pH 7.3 KRB (fig. 2a), and had decreased significantly after 45 (p=0.035) and 60 min (17.42±0.2 Ω·cm⁻²; p=0.003). When pH 7.2 KRB was used, RTM decreased from the 1st min (fig. 2a), and RTM was significantly decreased from 10 min (p=0.048) until 60 min (15.54±0.4 Ω·cm⁻²; p=0.002). The change in RTM was more

FIGURE 1. a) Transmesothelial resistance (RTM) after addition of Krebs' solution with different glucose concentrations to the pleural surface. Data are presented as mean±SEM, n=10 for each glucose concentration. ●: control; ■: Krebs' + 100 mg glucose; ▲: Krebs' + 40 mg glucose; ◆: Krebs' without glucose. *: p<0.05 versus control; #: p<0.05 between groups. b) Change in RTM after the 1st, 45th and 60th mins. ■: Krebs' + 100 mg glucose; ▲: Krebs' + 40 mg glucose; ◆: Krebs' without glucose.

FIGURE 2. a) Transmesothelial resistance (R_{TM}) after addition of Krebs' solution with different pH values to the pleural surface. Data are presented as mean $\pm$ SEM, $n=9$ for each pH. $\bullet$: control; $\blacksquare$: Krebs at pH 7.4; $\blacktriangle$: Krebs at pH 7.3 glucose; $\blacklozenge$: Krebs at pH 7.2. *: $p<0.05$ versus control; #: $p<0.05$ between groups. b) Change in R_{TM} after the 1st, 30th and 60th mins. $\blacksquare$: Krebs at pH 7.4; $\blacksquare$: Krebs at pH 7.3; $\blacksquare$: Krebs at pH 7.2.

intense for pH 7.2 KRB than for pH 7.4 ($p=0.010$) and pH 7.3 KRB ($p=0.008$) after 30 min ($3.76 \pm 0.2 \Omega\cdot\text{cm}^2$ versus $0.74 \pm 0.3 \Omega\cdot\text{cm}^2$; $p=0.005$) and after 60 min ($3.76 \pm 0.2 \Omega\cdot\text{cm}^2$ versus $2.64 \pm 0.2 \Omega\cdot\text{cm}^2$; $p=0.037$; fig. 2b).

Low-glucose and low-pH KRB

R_{TM} had decreased immediately and significantly 5 min after the addition of pH 7.2 KRB without glucose ($17.94 \pm 0.4 \Omega\cdot\text{cm}^2$; $p=0.049$; fig. 3a), after 30 min ($p=0.005$) and after 60 min ($14.23 \pm 0.4 \Omega\cdot\text{cm}^2$; $p=0.001$). The net R_{TM} decrease caused by pH 7.2 KRB was greater ($p=0.001$) than that caused by KRB without glucose (fig. 3b) 10 min after addition. The R_{TM} decrease caused by no-glucose pH 7.2 KRB was even greater ($p=0.002$ versus R_{TM} drop with no-glucose KRB; $p=0.937$ versus R_{TM} drop with pH 7.2 KRB) and this drop became significant from the 5th min after addition.

DISCUSSION

The main finding of the current study is that solutions with low glucose and/or low pH caused electrochemical alterations of the healthy human parietal pleura. PD T_M and R_{TM} were decreased, suggesting that the electrochemical transport ability of the pleura had increased [9]. The R_{TM} decrease was quicker

FIGURE 3. a) Transmesothelial resistance (R_{TM}) after addition of Krebs' solution with different glucose concentrations and/or pH levels to the pleural surface. Data are presented as mean $\pm$ SEM, $n=9$ for each glucose concentration. $\bullet$: control; $\blacklozenge$: Krebs' without glucose; $\blacktriangle$: Krebs' at pH 7.2; $\blacksquare$: Krebs' without glucose at pH 7.2. *: $p<0.05$ versus control; #: $p<0.05$ between groups. b) Change in R_{TM} after the 1st, 30th and 60th mins. $\blacksquare$: Krebs' without glucose; $\blacksquare$: Krebs' at pH 7.2; $\blacksquare$: Krebs' without glucose at pH 7.2.

and more intense as less glucose and lower-pH KRB were used. Lower pH was observed to be more potent than low glucose in altering the electrochemistry of the pleural membrane. Finally, the effects caused by low glucose and low pH seem to be additive.

The pleural membrane is considered to be "leaky", allowing solutes and substrates to be transported [8, 10]. Glucose entry to epithelial cells, apart from the glucose transporter system, is coupled with sodium *via* the sodium-glucose co-transporter [9]. This could provide an osmotic gradient, affecting overall tissue permeability [11, 12], which in the current study is also suggested by the observed decrease of PD T_M and R_{TM} [9]. It has been suggested that active electrolyte transportation across the pleural mesothelium involves Na^+/H^+ exchangers [8, 9] and thus pH could link the acidic environment with electrolyte balance within the pleural cavity.

Low glucose level and low pH are considered to be biochemical indicators of poor outcome or diagnostic indicators for undiagnosed pleural exudates [3, 6, 7, 13]. The results

of the present study suggest that a normal glucose content and pH could be important for pleural electrophysiological stability and thus low glucose concentrations or lowered pH in effusions are not only the result of but are also potent factors in further alteration of pleural function.

Lymphatic drainage impairment is believed to play a key role in exudate formation [14, 15]. Pleural capillary permeability alterations involve an increase in water and electrolyte transportation and macromolecule leakage [16]. Disease cells [1, 3], bacteria, inflammatory cells and mediators [1, 4] interact with the mesothelium causing altered selectivity, increased permeability [17] and decreased fluid removal [18], leading to further fluid accumulation and substrate imbalance. This substrate (glucose) imbalance can itself further change the pleural function. A more acidic pleural environment impairs the pleural function further, leading to a deteriorating cycle of further fluid accumulation, substrate imbalance and a shift to more acidic environment. In the present study, this process was greater and faster when greater substrate imbalance or a more acidic environment was simulated.

In conclusion, reduced glucose content and/or low pH of the pleural fluid could alter the progress of effusion by altering the function of the pleural membrane.

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