

Effect of Cimetidine on the Electrophysiologic Profile of Isolated Visceral Sheep Peritoneum

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The peritoneal mesothelium is a barrier to ion transport in peritoneal dialysis. Cimetidine is an H₂ receptor antagonist and a potent inhibitor of Na⁺/H⁺ antiporter, which is found in the plasma membranes of various cell types, including mesothelial cells. Recent reports linked Na⁺/H⁺ antiporter stimulation with increasing peritoneal fibroblast proliferation. The aim of the present study was to investigate by means of Ussing chamber experiments the effect of cimetidine on the transmesothelial electrical resistance (R_{TM}) of isolated visceral sheep peritoneum.

Peritoneal samples obtained from adult sheep were collected from the slaughterhouse and transferred in oxygenated Krebs–Ringer bicarbonate (KRB) solution to the laboratory within 30 minutes of the animal's death. The peritoneal tissue was transferred in a cooled KRB solution (4°C, pH 7.5) bubbled with 95% O₂/5% CO₂. A planar sheet of the visceral peritoneum was mounted in an Ussing-type chamber and cimetidine (10⁻³ mol/L) was added to the solution on the apical and basolateral sides. The R_{TM} was measured before and for 15 minutes serially after addition of the cimetidine. Results presented are the means ± standard error of the mean of 12 experiments.

Addition of cimetidine basolaterally induced, within 1 minute, an increase in the ΔR_{TM} of 35.97% ± 12.01% (p < 0.05), which returned to baseline after 15 minutes. The action of cimetidine on the apical side of the membrane was similar, with a rapid rise in the ΔR_{TM} of 47.3% ± 16.4% (p < 0.05) and a subsequent decline to control values.

The R_{TM} is inversely correlated with membrane permeability. The results of the present study indicate a rapid action of cimetidine on the permeability of visceral sheep peritoneum, probably through inhibition of mesothelial Na⁺/H⁺ antiporter. The increase in R_{TM} observed after addition of the cimetidine clearly indicates the existence of Na⁺/H⁺ antiporter on both sides of visceral sheep peritoneum. The clinical implications of our results should be further investigated.

Key words

Cimetidine, peritoneum, transmesothelial resistance, Ussing chamber

Introduction

The peritoneal mesothelium is one of the main barriers to water and ion transport from the peritoneal cavity to the peritoneal capillary bed (1). Physiologic solute transport across the peritoneal mesothelium is essential for effective peritoneal dialysis (PD) treatment. Peritoneal permeability to small solutes has been proved to increase with time on PD, a situation that eventually leads to ultrafiltration failure and PD dropout (2,3). Furthermore, glucose, the most commonly used osmotic agent in PD solutions, is known to have toxic effects on mesothelial cells (4).

Several studies performed in Ussing chambers have shown a clear association between transmesothelial electrical resistance (R_{TM}) and transcellular active ion transport in serosal membranes such as peritoneum (5–9), pleura (10,11), and pericardium (12). In those studies, permeability alterations were investigated in relation to the action on the membrane of substances such as sex hormones, insulin, channel blockers, NO inhibitors, catecholamines, opioids, and antibiotics and their metabolites.

Cimetidine is an H₂ receptor antagonist and a potent inhibitor of Na⁺/H⁺ antiporter (13). The latter

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is found in plasma membranes of various cell types, including mesothelial cells (13). Recent reports linked Na^+/H^+ antiporter stimulation with increasing peritoneal fibroblast proliferation (14) and increased transepithelial resistance in certain epithelia (15).

The aim of the present study was to investigate by means of Ussing chamber experiments the effect of cimetidine on the transmesothelial electrical resistance (R_{TM}) of isolated visceral sheep peritoneum.

Materials and methods

Intact sheets of visceral sheep peritoneum were obtained from the omentum of 12 adult sheep (male and female). The samples were collected from the slaughterhouse and transferred in oxygenated Krebs–Ringer bicarbonate (KRB) solution at 4°C to the laboratory within 30 minutes of the death of the animals. Immediately after removal, the peritoneal tissue was placed in KRB solution, which was balanced at pH 7.4 and bubbled with 95% O_2 /5% CO_2 . The solution contained 117.5 mmol/L NaCl, 1.15 mmol/L NaH_2PO_4 , 24.99 mmol/L NaHCO_3 , 5.65 mmol/L KCl, 1.18 mmol/L MgSO_4 , 2.52 mmol/L CaCl_2 , and 5.55 mmol/L glucose. The pieces of visceral peritoneum were obtained from the base of the greater omentum. Using a scalpel to remove the fat, they were carefully isolated from areas with underlying adipose tissue and were then examined for evidence of holes or adherent tissue by visual inspection. The surfaces of the tissue were touched as little as possible.

Specimens of visceral peritoneum 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 KRB solution, continuously oxygenated with 95% O_2 /5% CO_2 circulated by gas lift. Two pairs of Ag/AgCl electrodes monitored the transmesothelial potential difference (in millivolts) and the transmesothelial resistance (R_{TM} , in $\Omega\text{-cm}^2$) under open circuit conditions. The two parameters—potential difference and R_{TM} —were measured every 6 seconds under current clamp conditions. Experiments were conducted simultaneously in 3 PC-controlled chambers (Clamp software, version 2.14). Transmesothelial electrical parameters were measured in the basal state (that is, during an equilibration time of 30–40 minutes) and during incubation with cimetidine apically and basolaterally. After

addition of the cimetidine (10^{-3} mol/L), change in the R_{TM} was expressed as difference (ΔR_{TM}) from the baseline value. Because active transport of ions is influenced by temperature, all measurements were conducted with the apparatus held at 37°C.

The side of mesothelial cell membrane that *in vivo* faces the serosal fluid is here called the apical membrane, and the side facing the blood supply is called the basolateral membrane.

All solutions used were freshly prepared before each experiment, heated to 37°C and bubbled continuously with a 95% O_2 /5% CO_2 gas mixture. The results presented here are the mean of 12 separate experiments.

After the addition of cimetidine in each bathing solution (apical and basolateral consecutively), measurements were taken over a period of 15 minutes (at 1, 3, 5, 10, and 15 minutes). The voltage responses to applied current pulses of 50 μA amplitude and 200 ms duration were measured. The transmesothelial resistance was calculated by automatically deducting the resistance of the solution.

Statistical analysis was performed using Prism version 4.0 for Windows (GraphPad, La Jolla, CA, U.S.A.). All data are expressed as mean \pm standard error of the mean. The probability of error for comparison of the mean values was calculated using the *t*-test for paired data. Values of $p < 0.05$ were regarded as significant.

Results

The control R_{TM} (before addition of the cimetidine) was $21.8 \pm 0.51 \Omega\text{-cm}^2$. Addition of cimetidine apically induced, within 1 minute, a percentage increase in the R_{TM} (ΔR_{TM}) of $47.3\% \pm 16.4\%$ ($p < 0.05$), which returned to baseline after 15 minutes (Figure 1). The action of cimetidine on the basolateral side of the membrane was similar, with a rapid rise in ΔR_{TM} of $35.97\% \pm 12.01\%$ ($p < 0.05$) and a subsequent decline to control values (Figure 1). We observed no statistically significant differences in comparisons of the R_{TM} increases at 1 minute after addition of the cimetidine apically and basolaterally.

Discussion

In patients on PD, the functional integrity of the peritoneal membrane is pivotal to the success of the treatment. Understanding the physiology of the membrane is important for improving fluid ultrafiltration and optimizing solute removal.

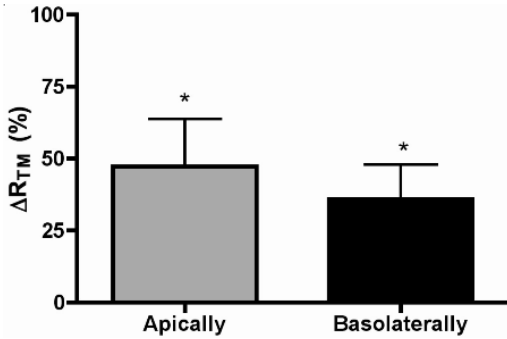


FIGURE 1 Comparison of the percentage change in transmesothelial electrical resistance (% ΔR_{TM}) of visceral sheep peritoneum 1 minute after the addition of cimetidine (10^{-3} mol/L) apically (grey bar) and basolaterally (black bar). Values are the mean and standard error of 12 experiments. * $p < 0.05$ versus control (baseline).

In the present work, we used recognized electrophysiologic techniques to study the transmesothelial resistance of visceral sheep peritoneal mesothelium. Electrical resistance is a measure of transmesothelial ionic permeability because electrical currents are carried by ions in aqueous solution.

The baseline R_{TM} values ($21.8 \pm 0.51 \Omega \cdot \text{cm}^2$) measured in this study lie within the values reported for “leaky” epithelial tissues such as renal proximal tubule, rabbit gallbladder, and sheep pleura (10). Addition of the cimetidine resulted in a significant increase in the R_{TM} of visceral peritoneum within 1 minute. Cimetidine acts as an inhibitor of Na^+/H^+ antiporter (13). The action of cimetidine resembles that of amiloride, another inhibitor of Na^+/H^+ antiporter. Previous studies by our group have clearly shown that the addition of amiloride in sheep and human peritoneum results in a similar rapid increase in R_{TM} (16,17); others have demonstrated that the characteristics of the inhibition of Na^+/H^+ antiporter by cimetidine and amiloride are very similar (13). It has also been confirmed that inhibition of any of the three isoforms of Na^+/H^+ antiporter (Nhe1, Nhe2, and Nhe3) may alter the electrical resistance of certain epithelial tissues such as the intestinal brush border, probably through a decrease in myosin II regulatory light chain (MLC) phosphorylation (15).

Phosphorylation of MLC is a requisite intermediate for an activated Na^+ -glucose co-transporter. The Na^+/H^+ antiporters and Na^+ -glucose

co-transporters are both expressed in mesothelial cells (13,16). The primary route of glucose absorption by peritoneal mesothelial cells is intracellular, with active Na^+ -glucose co-transport being one of the main molecular mechanisms for glucose uptake by those cells (18). Inhibition of glucose uptake by the mesothelial cells could have positive short-term effects by providing for a longer-lasting gradient in glucose concentration across the peritoneal membrane; it could also prove beneficial by inhibiting the long-term toxic effects of high glucose on peritoneal mesothelium (4).

The Na^+/H^+ antiporters may also be involved in the process of peritoneal fibrosis, which plays a pivotal role in the long-term functional integrity of the peritoneum and consequent survival of PD as a dialysis method. The Na^+/H^+ antiporters are activated by hypertonic glucose, acetate, and inflammation and may act as a transduction signal, increasing fibroblast proliferation and subsequently leading to extended peritoneal fibrosis and sclerosis (14). It may therefore be speculated that the use of Na^+/H^+ antiporter inhibitors such as amiloride and cimetidine could result in less damage to the peritoneal membrane during PD.

Conclusions

Our results indicate the presence of Na^+/H^+ antiporters in visceral peritoneal membrane. Inhibition of these antiporters by cimetidine has effects similar to those previously shown for amiloride. These electrophysiologic studies provide indirect evidence that cimetidine may possibly have beneficial effects in terms of glucose uptake by the mesothelial cells and peritoneal fibrosis; however, the clinical implications of our results should be further investigated.

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