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## Spirolactone Increases Permeability of Visceral Sheep Peritoneum

*Aldosterone is a key component of the renin-angiotensin-aldosterone system, and spironolactone, an aldosterone receptor blocker, shows beneficial effects in patients with end-stage renal disease and heart failure.*

*The aim of the present study was to investigate by means of Ussing chamber technique the effect of spironolactone on the transmesothelial permeability of visceral sheep peritoneum in vitro.*

*Peritoneal samples from the omentum of adult sheep were collected immediately after slaughter in a cooled and oxygenated Krebs-Ringer bicarbonate (KRB) solution. Isolated intact sheets of peritoneum were mounted in an Ussing-type chamber. Spirolactone ( $10^{-5}$  mol/L) was added apically and basolaterally to the KRB solution. The transmesothelial resistance ( $R_{TM}$ ) was measured before and serially for 30 minutes after the addition of the substances. Data present the mean  $\pm$  standard error of 6 experiments in each case.*

*The control  $R_{TM}$  was  $19.8 \pm 0.36 \Omega \cdot cm^2$ . The addition of spironolactone resulted in a reduction in the  $R_{TM}$ , which became significant on both sides of the membrane within 10 minutes and remained significantly different thereafter. The maximum reduction of  $R_{TM}$  ( $\Delta R_{TM}\%$ ) reached  $24.8\% \pm 2.3\%$  ( $p < 0.01$ ) apically and  $26.3\% \pm 3.2\%$  ( $p < 0.01$ ) basolaterally.*

*Our data clearly show that spironolactone increases the permeability of visceral sheep peritoneum in a lasting manner. Increased peritoneal permeability could result in increased sodium removal, which has acknowledged beneficial effects both in patients undergoing peritoneal dialysis and in patients with heart failure. Further clinical studies investigating the effect of spironolactone on sodium removal in peritoneal dialysis are justified.*

### Key words

Aldosterone, peritoneal permeability, renin-angiotensin-aldosterone system, spironolactone

### Introduction

In patients with end-stage renal disease (ESRD), heart failure is common and the rate of cardiovascular morbidity appears remarkably elevated compared with that in the general population. The renin-angiotensin-aldosterone system (RAAS) is responsible for maintaining blood pressure and extracellular volume, and is closely involved in the progression to ESRD and in the pathophysiology of congestive heart failure. Angiotensin blockade by either or both of angiotensin converting-enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) has proved not to be a complete renoprotective approach (1–3).

Aldosterone is another key component of RAAS. Aldosterone was originally believed to be important in the pathophysiology of heart failure merely by promoting sodium retention and potassium and magnesium loss. However, in the past several years, research has shown that it also causes myocardial and vascular fibrosis and damage (1–3).

Spirolactone (SLN) is a competitive inhibitor of aldosterone, which completely suppresses the effects of that mineralocorticoid hormone. It is also considered to be cardio- and renoprotective, because it may prevent fibrosis by blocking the effects of aldosterone on the formation of collagen (1–3). During peritoneal dialysis (PD), alterations in peritoneal function related to structural changes in peritoneal membrane, repeated episodes of bacterial peritonitis, and peritoneal fibrosis are common complications (4–6). Clinical and experimental data emphasize the essential contribution of the renin-angiotensin system in peritoneal fibrosis and PD (7,8). In animal models, SLN alone or in combination with an ACEI or ARB effectively reduced peritoneal fibrosis (9,10).

Several studies performed in Ussing chambers have shown a clear association between transmesothelial

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electrical resistance ( $R_{TM}$ ) and transcellular active ion transport in serosal membranes such as peritoneum (11–13) and pleura (14,15). The aim of the present study was to investigate the effect of SLN on the transmesothelial permeability of isolated visceral sheep peritoneum by means of Ussing chamber technique. The effect of SLN has never previously been investigated in this context.

### Materials and methods

Peritoneal samples from the base of the greater omentum of adult sheep were collected immediately after the animals were euthanized. The samples were transferred directly from the slaughterhouse to the laboratory within 30 minutes in oxygenated Krebs–Ringer bicarbonate (KRB) solution at 4°C. The KRB solution contained 117.5 mmol/L NaCl, 1.15 mmol/L  $\text{NaH}_2\text{PO}_4$ , 24.99 mmol/L  $\text{NaHCO}_3$ , 5.65 mmol/L KCl, 1.18 mmol/L  $\text{MgSO}_4$ , 2.52 mmol/L  $\text{CaCl}_2$ , and 5.55 mmol/L glucose. The solution was balanced at pH 7.4 and bubbled with 95%  $\text{O}_2$ /5%  $\text{CO}_2$ . The visceral peritoneum specimens were isolated by detaching them carefully from the underlying adipose tissue using a scalpel to remove fat. The pieces of peritoneum were then visually examined for holes and adherent tissue. During the entire experiment, precautions were taken to avoid touching the surface of the samples.

Intact and planar sheets of the visceral sheep peritoneum were then carefully mounted in Ussing chambers (Dipl.–Ing. K. Mussler Scientific Instruments, Aachen, Germany) with an open surface area of 1 cm<sup>2</sup>. Tissues were bathed with 4 mL of KRB solution on each side of the membrane, continuously oxygenated with 95%  $\text{O}_2$ /5%  $\text{CO}_2$  circulated by gas lift. Two pairs of Ag/AgCl electrodes monitored the transmesothelial potential difference (in millivolts) and the transmesothelial resistance ( $R_{TM}$ ,  $\Omega \cdot \text{cm}^2$ ), under open circuit conditions. Those two parameters were measured every 6 seconds under current clamp conditions. Experiments were conducted simultaneously in up to 4 chambers controlled by a personal computer (Clamp software, version 2.14).

The experimental solution bathing the surface of the peritoneum that *in vivo* faces the peritoneal fluid is called the serosal solution; the solution bathing the surface that *in vivo* is exposed to the blood supply is called the mucosal solution. The mesothelial cell membrane facing the fluid is called the apical side, and that facing the blood is called the basolateral side.

Transmesothelial electrical parameters were measured in the basal state (after an equilibration time of 30 – 40 minutes) and during incubation with SLN apically and basolaterally. After the addition of the KRB solution containing SLN ( $10^{-5}$  mol/L) to the serosal and mucosal solutions, changes in the  $R_{TM}$  were expressed as the difference from the baseline value. Because active transport of ions is influenced by temperature, transmesothelial electrical parameters were all measured at 37°C. All solutions were freshly prepared before each experiment, heated to 37°C, and bubbled continuously with a 95%  $\text{O}_2$ /5%  $\text{CO}_2$  gas mixture. The results presented here are the mean of 6 separate experiments in each case.

After the addition of the compound to each bathing solution (mucosal or serosal), the voltage response to applied current pulses of 50  $\mu\text{A}$  amplitude and 200 ms duration was measured for 30 minutes at given intervals (1, 3, 5, 10, 15, 20, 30 minutes). The  $R_{TM}$  was calculated by the software, automatically deducting the baseline resistance of the solution.

Statistical analysis was performed using Instat 3 (GraphPad, San Diego, CA, U.S.A.). All data are expressed as mean  $\pm$  standard error of the mean. Statistical calculations and the probability of error for comparison of the mean values were performed by one-way or two-way analysis of variance (ANOVA) with Bonferroni post-test correction. Values of  $p$  less than 0.05 were considered significant.

### Results

The control  $R_{TM}$  (before the addition of SLN) was  $19.8 \pm 0.36 \Omega \cdot \text{cm}^2$ . The addition of SLN resulted in a reduction in the  $R_{TM}$  of the visceral peritoneum, which became significant on both sides within the first 10 minutes and remained significant thereafter (Figure 1).

The maximum  $\% \Delta R_{TM}$  reduction both apically and basolaterally was registered at the 20th minute after addition of the SLN, and reached  $24.8\% \pm 2.3\%$  apically [ $p < 0.01$ , Figure 1(a)] and  $26.3\% \pm 3.2\%$  [ $p < 0.01$ , Figure 1(b)] basolaterally.

No statistically significant differences were observed in comparisons of apical and basolateral percentage reductions of the  $R_{TM}$  at any time point.

### Discussion

In the present study, we used recognized electrophysiologic techniques to investigate the properties of visceral peritoneal mesothelium from sheep. Use of

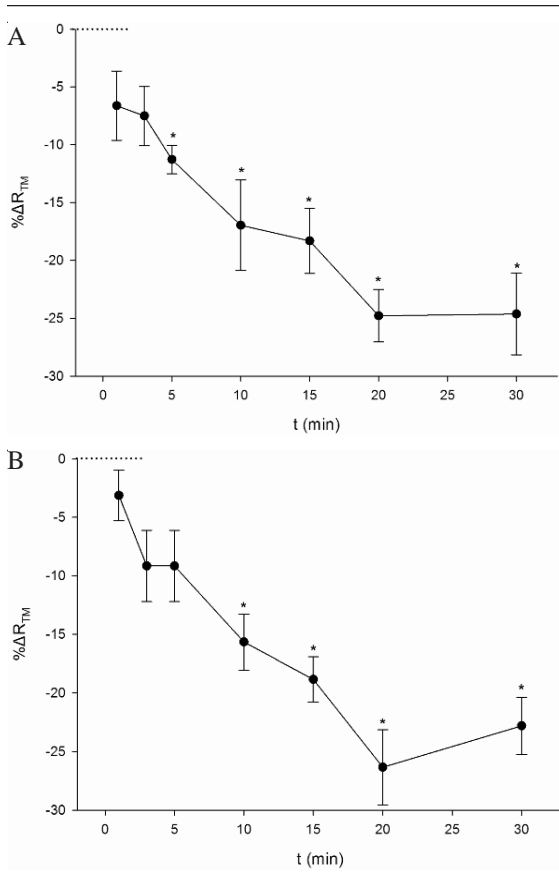


FIGURE 1 Time course of the percentage reduction in transmesothelial electrical resistance (% $\Delta R_{TM}$ ) of visceral sheep peritoneum in relation to the control resistance ( $R_{TM}$ , dotted line at 0%) after addition of spironolactone  $10^{-5}$  mol/L at 0 min (A) apically and (B) basolaterally. Values are the mean and standard error of 6 experiments. \*  $p < 0.01$ .

these techniques allowed us to evaluate two important parameters: transmesothelial potential difference and transmesothelial resistance. The potential difference across the mesothelium suggests the presence of net ion transport (16). Electrical resistance is a measure of transmesothelial ionic permeability because electrical currents are carried by ions in aqueous solution. A clear association between  $R_{TM}$  and active ion transport has been shown in previous studies (13,14).

Our data exhibit a rapid reduction of the transmesothelial resistance of visceral sheep peritoneum both apically and basolaterally. This rapid effect, occurring within 10 minutes, suggests the involvement of SLN in a nongenomic pathway. The rise in ion transport across the peritoneal membrane is

probably a result of the activation of sodium channels that have been previously reported to exist in peritoneal (12,17) and pleural (14,15) mesothelium. In an electrophysiologic study of rat distal colon, co-administration of SLN inhibited the effects of aldosterone on  $Na^+$  and  $K^+$  transport (18). Many studies, two recent, implicate a central involvement of SLN in the regulation of epithelial sodium channels. However, this specific effect is mediated only through a genomic pathway (19,20).

Our finding, at first reading, might seem to contradict knowledge of the function of a mineralocorticoid inhibitor such as SLN. However, the nongenomic effect of aldosterone and its inhibitors on plasma membrane  $Na^+-K^+$  adenosine triphosphatase (ATPase) might be a possible explanation. Specifically, it has recently been shown that aldosterone has a significant nongenomic effect on  $Na^+-K^+$  ATPase, mediated through protein kinase C activation. This effect is inhibited by eplerenone, a new mineralocorticoid receptor antagonist (21). In the end, the suggested increase in the permeability of the peritoneal membrane after the addition of SLN could result in increased sodium removal.

In ESRD, the development of hyperkalemia poses a therapeutic dilemma (22). In the Randomized Aldactone Evaluation Study, patients with severe left ventricular dysfunction experienced a 30% – 35% reduction in the risk of overall mortality, cardiac mortality, and hospitalization (3), but patients with significant renal failure were excluded. Various studies are trying to address this issue (23,24). Those studies imply that SLN can be used safely in carefully selected and closely monitored patients.

## Conclusions

It is evident that SLN increases, in a rapid and lasting fashion, the transmesothelial permeability of visceral sheep peritoneum *in vitro*. This nongenomic action might be the result of sodium removal, a beneficial effect for PD patients in particular. More studies are needed to elucidate the physiologic role and particularly the clinical implications of these findings.

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