The peritoneal mesothelium is a biologic barrier to water and ion transport. Its functional and structural integrity is crucial for peritoneal dialysis treatment. In vivo studies have shown that corticosteroids increase transcellular water transport and ultrafiltration of the rat peritoneum.

In the present study, we used Ussing chamber technique to investigate the effect of dexamethasone on the transmesothelial permeability of the visceral sheep peritoneum in vitro.

Peritoneal samples from the omentum of adult sheep were collected in a cooled and oxygenated Krebs–Ringer bicarbonate (KRB) solution immediately after the death of the animals. Isolated intact sheets were mounted in an Ussing-type chamber. Dexamethasone (10⁻⁶ mol/L) and its inhibitor mifepristone (10⁻⁵ mol/L) were added apically and basolaterally, alone and in combination to the KRB solution. The transmesothelial resistance (R<sub>TM</sub>) was measured for 1 hour before and serially after the addition of the substances. Data are expressed as mean ± standard error of 6 experiments in each case.

The control R<sub>TM</sub> was 21.5 ± 0.42 Ω•cm². Dexamethasone induced a significant reduction of R<sub>TM</sub> within 15 minutes, which continued for the entire experiment. The maximum effect (%ΔR<sub>TM</sub>) was observed at 30 – 60 minutes after the addition of dexamethasone apically 46.2% ± 7.14% (p < 0.01) and basolaterally 35.3% ± 7.76% (p < 0.01). Mifepristone acted as an agonist on both sides of the membrane and significantly inhibited the dexamethasone effect.

Our findings clearly indicate that dexamethasone rapidly increases the transmesothelial permeability of visceral sheep peritoneum. The rapid effect implicates dexamethasone and probably mifepristone as being involved in a common nongenomic pathway. Further investigation is necessary to elucidate the underlying mechanisms and perspectives of these findings.

Key words
Dexamethasone, mifepristone, peritoneal permeability, Ussing chamber

Introduction
In peritoneal dialysis (PD), the peritoneum functions as a semipermeable membrane that regulates the selective transport of water and solutes between the systemic circulation and the peritoneal cavity (1). The peritoneal mesothelium is an important biologic barrier, and the mesothelial cells provide the first line of defense during long-term exposure to unphysiologic PD solutions and against micro-organisms during infective peritonitis. The efficacy of PD treatment depends on the integrity of the peritoneal mesothelium and is reflected by ultrafiltration (UF) adequacy. One of the major problems associated with PD is UF failure, which can affect up to 50% of PD patients treated for more than 6 years. Disturbance of peritoneal permeability leads to UF failure and inadequacy of therapy (2,3).

To this end, improvement of PD solutions by adding pharmacologic agents capable of maintaining osmotic gradient across the peritoneal membrane without affecting its consistency is an intriguing field of research. Dexamethasone is a synthetic glucocorticoid receptor agonist known for its anti-inflammatory activity. Previous in vivo studies in rats showed that dexamethasone improved water permeability and UF by inducing the expression of aquaporin-1 in the peritoneal membrane, without affecting the osmotic gradient and the permeability for small solutes (4,5).

Several studies performed in Ussing chambers have shown a clear association between transmesothelial
electrical resistance \( R_{\text{TM}} \) and the transcellular active ion transport in serosal membranes such as the peritoneum (6–8) and the pleura (9,10). In those studies, the permeability alterations of the actual membrane were investigated in relation to the action of certain substances such as insulin, antibiotics, NO inhibitors, and sex hormones.

The aim of the present study was to investigate the effect of dexamethasone on the transmesothelial permeability of isolated visceral sheep peritoneum by means of Ussing chamber technique. In this context, the effect of dexamethasone has never previously been investigated.

Materials and methods

Peritoneal samples from the base of the greater omentum of adult sheep were collected immediately after the deaths of the animals and were directly transferred from the slaughterhouse to the laboratory in oxygenated Krebs–Ringer bicarbonate solution (KRB) at 4°C within 30 minutes. The KRB solution contained 117.5 mmol/L NaCl, 1.15 mmol/L NaH₂PO₄, 24.99 mmol/L NaHCO₃, 5.65 mmol/L KCl, 1.18 mmol/L MgSO₄, 2.52 mmol/L CaCl₂, and 5.55 mmol/L glucose. The solution was balanced at pH 7.4 and bubbled with 95% O₂/5% CO₂. The visceral peritoneum specimens were isolated by carefully detaching them from the underlying adipose tissue using a scalpel. The pieces thus obtained were then visually examined for holes and adherent tissue. During the entire experiment, precautions were taken to avoid touching the membrane surface.

Intact and planar sheets of the visceral sheep peritoneum were carefully mounted in Ussing chambers (Dipl.-Ing. K. Mussler Scientific Instruments, Aachen, Germany) with an open surface area of 1 cm². Tissues were bathed with 4 mL of KRB solution on each side of the membrane, continuously oxygenated with 95% O₂/5% CO₂. The visceral peritoneum specimens were isolated by carefully detaching them from the underlying adipose tissue using a scalpel. The pieces thus obtained were then visually examined for holes and adherent tissue. During the entire experiment, precautions were taken to avoid touching the membrane surface.

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

Results

The control \( R_{\text{TM}} \) (before addition of DEX) was 21.5 ± 0.42 Ω · cm². After 15 minutes, DEX (10⁻⁶ mol/L) induced a gradual and significant reduction of the \( R_{\text{TM}} \) of the visceral peritoneum. The maximum percentage \( \Delta R_{\text{TM}} \) reduction was registered 30 – 60 minutes after addition of DEX both apically and basolaterally, reaching 46.2% ± 7.14% \( [p < 0.01, \text{Figure 1(A)}] \) and 35.3% ± 7.76% \( [p < 0.01, \text{Figure 1(B)}] \), respectively.

The combination of DEX plus the inhibitor of glucocorticoid receptors RU486 (10⁻⁵ mol/L) when applied
both apically (24.39% ± 4.44%) and basolaterally (18.71% ± 7.16%), resulted in a partial inhibition of the DEX effect. However, the observed effect was significant compared with baseline (Figure 2). When RU486 was added alone, it exhibited an agonistic effect, but on both sides it induced a milder effect that did not differ greatly from the effect exerted by its combination with DEX (Figure 2).

**Discussion**

In the present study, we used recognized electrophysiologic techniques to investigate the ionic resistance and transmengial potential of sheep visceral peritoneal mesothelium. Use of these techniques allowed us to evaluate two important parameters: transmengial potential difference and transmengial resistance. The potential difference across the mesothelium suggests the presence of net ion transport (11). Electrical resistance is a measure of transmengial ionic permeability because electrical currents are carried by ions in aqueous solution. A clear association between $R_{TM}$ and active ion transport was shown in previous studies (8,10). Our data show a rapid reduction of transmengial resistance of the visceral peritoneum after the addition of DEX both apically and basolaterally. This rapid effect, within 15 minutes, implicates a nongenomic pathway in this DEX effect. A rapid, nongenomic steroid action of DEX has been identified in several tissues such as human bronchial epithelial cells, human endometrial cells, and rabbit and rat distal colon (12–16). In addition, DEX has been shown to stimulate net Na$^+$ transport and water absorption in rat distal colon and bovine mammary epithelium (17,18). It was also found to increase the activity of Na$^+$–K$^+$–ATPase in alveolar type II cells by inducing its translocation from intracellular pools to the plasma membrane (19). Those findings might explain the gradual reduction of $R_{TM}$ (corresponding to an increase in ionic permeability) induced by DEX in our experiments.

**FIGURE 1** The time-course percentage reduction of transmengial electrical resistance ($\Delta R_{TM}$) of the visceral peritoneum in relation to the control $R_{TM}$ after addition of dexamethasone (10$^{-6}$ mol/L) (A) apically and (B) basolaterally. Values are mean and standard error of 6 experiments each. ♦ p < 0.05. ♦♦ p < 0.01.

**FIGURE 2** The maximum percentage reduction of the transmengial electrical resistance ($\Delta R_{TM}$) of the visceral peritoneum after addition of dexamethasone [DEX (10$^{-6}$ mol/L)], mifepristone [RU486 (10$^{-5}$ mol/L)], and a combination of the two substances, apically and basolaterally. Values are mean and standard error of 6 experiments each. * p < 0.05 versus control (baseline). † p < 0.05 for DEX vs. RU486+DEX, apically and basolaterally.
Mifepristone partially inhibits the effect of DEX. The fact that RU486 exerts agonistic activity could support the hypothesis of a common nongenomic mechanism of action with DEX. Numerous studies in the past decade have reported the partial agonist activity of RU486 in some cell types. A recent publication suggests that this action is dependent on the concentration of glucocorticoid receptors in the cell (20). The nongenomic mechanism mediating the action of RU486 has already been confirmed in human myometrium in vitro. Namely, RU486 was shown (21) to act via modulation of the second messenger system (protein kinase A and cAMP). Glucocorticoids may play an important role in the future progress toward long-lasting and better UF with PD solutions.

In vivo experiments and clinical trials are already evaluating the beneficial effects of glucocorticoids in PD patients (22–24).

Conclusions

It is evident that DEX acts rapidly on visceral sheep peritoneum in vitro, increasing its transmesothelial permeability by a nongenomic pathway. Mifepristone inhibits the effect of DEX, but also acts as a partial agonist. The rapid effect implicates DEX and probably RU486 as being involved in a common nongenomic pathway. More studies are needed to elucidate the physiologic role represented by these findings and particularly their clinical implication.

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