

Rapid Effect of Dexamethasone on the Permeability of Visceral Sheep Peritoneum

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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_{TM}) was measured for 1 hour before and serially after the addition of the substances. Data are expressed as mean \pm standard error of 6 experiments in each case.

The control R_{TM} was $21.5 \pm 0.42 \Omega \cdot \text{cm}^2$. Dexamethasone induced a significant reduction of R_{TM} within 15 minutes, which continued for the entire experiment. The maximum effect ($\% \Delta R_{TM}$) was observed at 30 – 60 minutes after the addition of dexamethasone apically $46.2\% \pm 7.14\%$ ($p < 0.01$) and basolaterally $35.3\% \pm 7.76\%$ ($p < 0.01$). 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

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electrical resistance (R_{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 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 solution was balanced at pH 7.4 and bubbled with 95% O_2 /5% CO_2 . 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% 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} ($\Omega \cdot \text{cm}^2$)] under open circuit conditions. Those two parameters were measured every 6 s under current clamp conditions. Experiments were conducted simultaneously in a maximum of 6 chambers controlled by a personal computer (Clamp software, version 2.14: AC Micro-Clamp, Aachen, Germany).

The experimental solution bathing the surface of the peritoneum that *in vivo* faces the peritoneal fluid is here called the serosal solution; the solution bathing

the surface that *in vivo* is exposed to the blood supply is here called the mucosal solution. The mesothelial cell membrane facing the fluid side of the peritoneal cavity is here called the apical membrane side, and that facing the blood side is here called the basolateral membrane side.

Transmesothelial electrical parameters were measured in the basal state (after an equilibration time of 30–40 minutes) and during incubations with dexamethasone (DEX), mifepristone (RU486), and a combination of the two substances apically and basolaterally. After the addition of the KRB solution to the serosal and to the mucosal solutions containing DEX (10^{-6} mol/L), RU486 (10^{-5} mol/L), and a combination, 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% O_2 /5% CO_2 gas mixture. The results presented here are the mean of 6 separate experiments in each case.

After the addition of the DEX and RU486 alone or in combination in each bathing solution (mucosal or serosal), the voltage response to applied current pulses of 50 μA amplitude and 200 ms duration was measured for 1 hour at selected times (1, 3, 5, 10, 15, 20, 30, 40, 50, 60 minutes). The R_{TM} was calculated by the software, automatically deducting the resistance of the solution.

Statistical analysis was performed with Instat 3 (GraphPad, San Diego, CA, U.S.A.). All data are expressed as mean \pm 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_{TM} (before addition of DEX) was $21.5 \pm 0.42 \Omega \cdot \text{cm}^2$. After 15 minutes, DEX (10^{-6} mol/L) induced a gradual and significant reduction of the R_{TM} of the visceral peritoneum. The maximum percentage ΔR_{TM} reduction was registered 30–60 minutes after addition of DEX both apically and basolaterally, reaching $46.2\% \pm 7.14\%$ [$p < 0.01$, Figure 1(A)] and $35.3\% \pm 7.76\%$ [$p < 0.01$, Figure 1(B)], respectively.

The combination of DEX plus the inhibitor of glucocorticoid receptors RU486 (10^{-5} mol/L) when applied

both apically ($24.39\% \pm 4.44\%$) and basolaterally ($18.71\% \pm 7.16\%$), 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 transmesothelial potential of sheep visceral peritoneal mesothelium. Use of 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 (11). 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 was shown in previous studies (8,10). Our data show a rapid reduction of transmesothelial 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 $\text{Na}^+-\text{K}^+-\text{ATPase}$ 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.

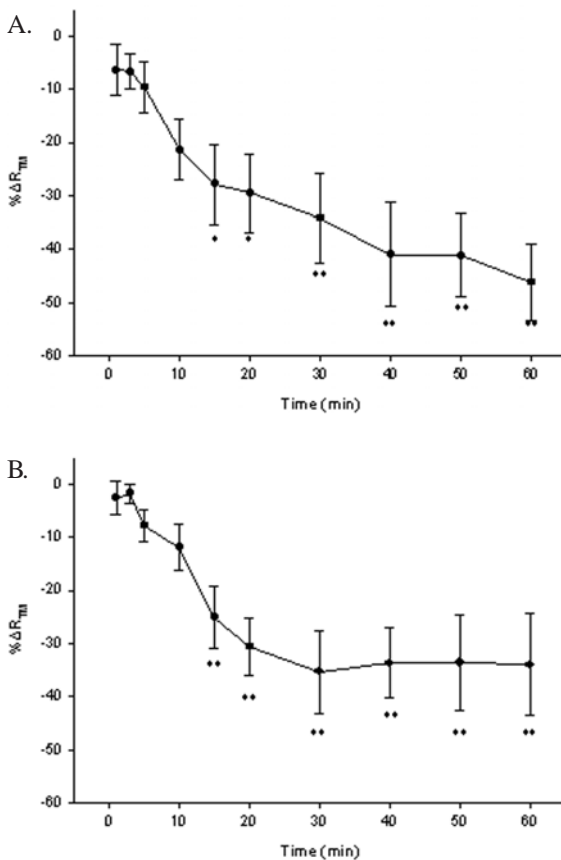


FIGURE 1 The time-course percentage reduction of transmesothelial electrical resistance (ΔR_{TM}) of the visceral peritoneum in relation to the control R_{TM} after addition of dexamethasone (10^{-6} mol/L) (A) apically and (B) basolaterally. Values are mean and standard error of 6 experiments each.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

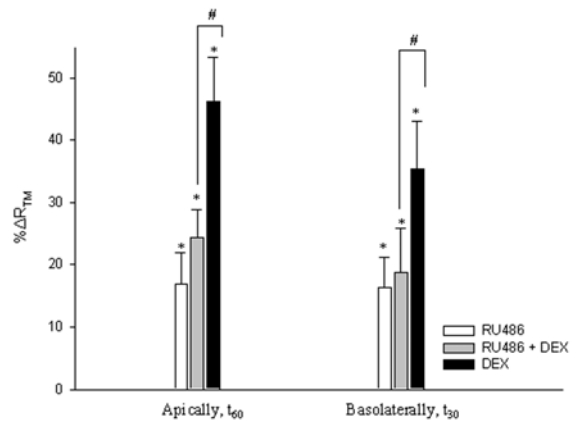


FIGURE 2 The maximum percentage reduction of the transmesothelial electrical resistance (ΔR_{TM}) of the visceral peritoneum after addition of dexamethasone [DEX (10^{-6} mol/L)], mifepristone [RU486 (10^{-5} mol/L)], and a combination of the two substances, apically and basolaterally. Values are mean 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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