Influence of Prednisolone on Glucose and Uric Acid Transport Across Peritoneal Membrane In Vitro

Prednisolone and other glucocorticosteroids are used by some peritoneal dialysis patients because of underlying diseases such as peritonitis. Although corticosteroids are potent inhibitors of various processes during inflammation, their influence on the transport properties of peritoneum is little known. Our study investigated the influence of prednisolone (0.001 g/dL) on glucose (1.8 g/dL) and uric acid (0.02 g/dL) transfer across isolated parietal peritoneum taken from the anterior abdominal wall of white Hyplus 59 rabbits and placed inside a modified Ussing-type chamber. Values for transfer from the interstitial (I) to the mesothelial (M) side of membrane (I→M) and in the opposite direction (M→I) were calculated using the mathematical model of mass transport and are expressed as a coefficient of diffusive permeability [P (in centimeters per second)].

Four separate series of experiments were done. In the first and second series, we respectively examined glucose transport under control conditions (for 120 minutes) and then before (15 – 60 minutes) and after (75 – 120 minutes) introduction of prednisolone on the M side of the membrane. In the third and fourth series, similar studies of uric acid transfer were done. In the control (first and third) series, the stability of bidirectional transport for solute of interest was observed. The values of P ± standard error of the mean (all ×0.0001) for I→M and M→I transfer of glucose were, respectively, 2.489 ± 0.329 cm/s and 2.259 ± 0.493 cm/s. In the case of uric acid, the transport values were lower and amounted 1.936 ± 0.324 cm/s and 1.895 ± 0.596 cm/s for I→M and M→I respectively. Application of prednisolone on the M side of membrane lowered bidirectional transfer of glucose across peritoneal membrane by a mean of 73% (p < 0.02) and transport of uric acid by a mean of 19% (p < 0.003).

These results show that, in vitro, prednisolone lowers glucose and uric acid transport across the peritoneal membrane, modifying the transfer dynamics of glucose to a greater extent. These observations may have clinical importance, especially in patients with disorders of peritoneal permeability, diabetes, and hyperuricemia.

Key words
Transperitoneal transport, prednisolone, glucose, uric acid

Introduction
Peritoneal dialysis (PD) is used as a renal replacement therapy in end-stage renal disease. The relatively large surface area of the semipermeable peritoneum (the largest serous membrane in the body) is effectively used to remove water and uremic toxins from the body fluids of patients and to administer drug therapy (1). In most countries, glucose is used to create a crystalloid osmotic pressure gradient across the peritoneal membrane. In standard fluids applied during PD, highly concentrated glucose solutions (1.36 – 3.86 g/dL—that is, 15 – 40 times the physiologic concentration) are used to develop sufficient ultrafiltration during therapy. However, as dwell time increases, ultrafiltration decreases because of rapid glucose absorption from the peritoneal cavity to the vascular bed, with a resulting decline in the osmotic pressure gradient. This glucose absorption changes during peritonitis and long-term dialysis therapy as a consequence of structural modifications of the membrane (2).

Elevated serum uric acid independently increases the risk for kidney disease (3). Hyperuricemia appearing in some PD patients is significantly associated with the rate of decline of residual renal function (4),
but its effect in end-stage renal disease has not yet been thoroughly elucidated. Uric acid is a marker of oxidative stress, and it may have a potential therapeutic role as an antioxidant. On the other hand, a strong reducing substance such as uric acid can also act as a pro-oxidant, particularly at elevated levels (5).

Peritonitis is a major complication of PD and one of the main reasons that patients are transferred to hemodialysis (6). Encapsulating peritoneal sclerosis is a rare but life-threatening complication of long-term PD. One treatment option (in both the inflammatory and fibrotic phases of encapsulating peritoneal sclerosis) is the use of low- or high-dose glucocorticosteroids (GKs)—among them, prednisolone. Unfortunately, GKs also produce serious side effects connected with genomic and nongenomic mechanisms that limit their use (7–9).

In human peritoneum, glucocorticoid nuclear receptors are highly expressed (10). Information about membrane receptors in peritoneal structures is lacking, and little is known about the effect of prednisolone on transperitoneal transfer properties. The aim of the present in vitro study was to verify the role of prednisolone in the transfer of glucose and uric acid through the peritoneal membrane.

**Methods**

The experiments used fragments of parietal peritoneum obtained from the abdominal wall of Hyplus 59 male rabbits, which were placed into a modified Ussing chamber system providing an active membrane surface area amounting to 1.1 cm². The study was approved by the Local Ethics Committee for Animal Research in Poznań, Poland (approval no. 47/2009). The chamber was connected by a peristaltic pump to a fluid reservoir containing 13 mL or 15 mL of Hanks solution of the following composition: NaCl, 136.88 mmol/L; KCl, 5.36 mmol/L; NaHCO₃, 4.16 mmol/L; CaCl₂, 1.26 mmol/L; KH₂PO₄, 0.44 mmol/L; Na₂HPO₄×12 H₂O, 0.34 mmol/L; MgCl₂×7 H₂O, 0.41 mmol/L. The solution was circulated at a rate of 11 mL/min. A constant pH of 7.4 and adequate oxygen content were maintained in the medium by continuous bubbling with a gas mixture of 5% CO₂ and 95% O₂. The entire system was placed into a thermostatic box at 37°C (11).

We determined the diffusion rate of glucose [initial concentration gradient: 1.8 g/dL; molecular weight: 180 Da (Polfa Tarchomin, Warsaw, Poland)] and uric acid [0.02 g/dL, 168 Da (Serva, Heidelberg, Germany)] from the mesothelial (M) to the interstitial (I) side of the peritoneal membrane and in the opposite direction. For each molecule, 2 separate series of transfer analyses were carried out:

- in control conditions without prednisolone (120 minutes), and
- before (15 – 60 minutes) and after (75 – 120 minutes) application of prednisolone [0.001 g/dL, 360 Da (PPH Galfarm, Krakow, Poland)] on the M side of the membrane.

Sampling of the medium was carried out at regular 15-minute intervals. The osmolality of the solutions amounted to 400 mOsm/kg H₂O for the glucose solution and 300 mOsm/kg H₂O for the uric acid solution. Glucose was measured using glucose oxidase (Cormay, Krakow, Poland). The concentration of uric acid in samples was assayed using uricase and peroxidase (Cormay, Krakow, Poland). Prednisolone (0.001 g/dL) did not interfere with either assay method.

A mathematical model of mass transport was used to estimate a diffusive permeability coefficient, $P$, for the study specimens (scaled to the surface area of the investigated membrane). The changes of $P$ attributable to the experimental modifications were determined individually for each experiment as a percentage of the control value before the modification and are presented as mean ± standard error of the mean for the whole series. In this way, for each membrane fragment, the initial part of the experiment served as a control for the second part (12). For the statistical analysis, we used the Statistica 8 software program (StatSoft, Tulsa, OK, U.S.A.). We applied the Wilcoxon test for paired data. A Shapiro–Wilks test was applied to evaluate data distributions. A value of $p < 0.05$ was considered statistically significant.

**Results**

In control conditions, the rate of glucose transfer was stable for 120 minutes of the experiment, and no differences were observed for transport directed from the I to the M side of membrane or in the opposite direction (Figure 1). In the control series, the values of $P$ (all $<0.0001$) were $2.489 ± 0.329$ cm/s for I→M transfer and $2.259 ± 0.493$ cm/s for M→I transfer. In the case of uric acid, the rate of transport was stable for 120 minutes (Figure 1), but the $P$ was lower,
amounting to 1.936 ± 0.324 cm/s for I→M transfer and 1.895 ± 0.596 cm/s for M→I transfer. Application of prednisolone on the M side of membrane diminished bidirectional glucose transfer across the peritoneal membrane by a mean of 73% (p < 0.02) and transport of uric acid by a mean of 19% (p < 0.003, Figure 2). The changes in glucose and uric acid transfer were already observed after 15 minutes, in the case of both I→M and M→I transport.

**Discussion**

In the present study, we analyzed the passage of glucose and uric acid across barriers including mesothelium, interstitium, and stagnant fluid layers (11). All solute transport during the 120 minutes of the control experiments was stable. Hexoses are small (molecular weight: 180 Da), neutral solutes with significant osmotic properties. Higher diffusion was observed for glucose (a hexose) than for uric acid (168 Da), despite the larger molecular weight of glucose. The difference is connected to the concentration gradient (1.8 g/dL vs. 0.002 g/dL) and the charge (neutral vs. negative) of those two solutes and is probably disproportionate because of the participation of active transport during hexose transfer. Assuming that transperitoneal passage of small solutes is mainly diffusive (by intercellular and transcellular pathways), it is nevertheless impossible to exclude active transport (2).

The influence of prednisolone on transperitoneal transfer is little known. This GK, a strong lipophilic solute, can easily and quickly penetrate lipid structures of the cell membrane. Prednisolone is responsible for changing physicochemical properties—for example, the regulation of membrane ion channels for calcium, sodium, potassium, and chloride in genomic and nongenomic mechanisms (13–15).

All the effects of GKs are considered to be mediated by four different mechanisms of action. Besides the classical genomic mechanism managed by the cytosolic glucocorticoid receptor (cGKCR), secondary nongenomic effects initiated by cGKCR, nongenomic effects mediated by membrane-bound glucocorticoid receptor (mGKCR), and nonspecific nongenomic effects caused by interactions with cellular membranes.
can be considered (13,14). The genomic mechanism is, among other things, connected with repression of vascular endothelial growth factor expression (16), and dialysate levels of vascular endothelial growth factor have been associated with higher transport and poorer ultrafiltration during PD (17). As an example of a nongenomic effect, GKs interact directly with enzymatic protein of cell membrane, influencing Ca^{2+} ATPase activity (13,14). The genomic action of GKs was observed after 30 minutes in a previous study, and their nongenomic action was characterized by rapid onset (seconds to minutes) and short duration (60 – 90 minutes) (18).

We cannot exclude the specific influence of a nongenomic mechanism of high-dose prednisolone (0.001 g/dL) on isolated peritoneal membrane. After addition of prednisolone in our study, significant changes in the transperitoneal passage of glucose and uric acid was observed after 15 minutes (which probably excludes genomic action at that stage). The reasons for the greater modifications in the case of glucose are difficult to determine. It might be connected with the higher participation of active transport in transperitoneal glucose transfer than in uric acid transfer. In an in vitro analysis using peritoneal mesothelial cells, messenger RNA expression for glucose transporters 1 and 3 (GLUT1, GLUT3) and glucose uptake were induced by high ambient hexose concentrations (in the same range as used in present study) (19). In the case of uric acid transport, data on similar correlations is lacking. Prednisolone probably modified mainly the active passage of glucose, which requires energy from ATP. Rapid nongenomic inhibition of ATP-induced Cl\(^{-}\) secretion by glucocorticoid was observed in human bronchial epithelium (13). Moreover, in our previous in vitro study, prednisolone (0.001 g/dL and 0.002 g/dL) lowered the resistance of colonic membrane after 20 minutes. The observed changes were dose dependent (20), which is characteristic in the case of both genomic and nongenomic mechanisms (18). Similarly, the effects of dexamethasone on the resistance of isolated visceral peritoneum were not inhibited by a specific blocker of glucocorticoid receptor, which demonstrates nongenomic action (15).

Reduction of glucose transfer during PD is beneficial from the clinical point of view. High glucose absorption from dialysis fluids might influence a number of metabolic imbalances—increased oxidative stress, altered adipokine levels, hyperinsulinemia, dyslipidemia—that are involved in determining PD patient survival (21). By contrast, a decrease in uric acid transfer across the peritoneal membrane is not advantageous to PD patients. Hyperuricemia increases the risk of hypertension and cardiovascular mortality during continuous ambulatory PD (22).

**Conclusions**

In vitro, prednisolone lowered glucose and uric acid transport across rabbit peritoneal membrane, modifying the transfer dynamics of glucose to a greater extent. The fast onset of the observed changes suggests participation of a nongenomic action of prednisolone in glucose and uric acid transfer. Those observations may have clinical importance, especially in PD patients with disorders of peritoneal permeability, diabetes, and hyperuricemia.

**Disclosures**

The authors have no financial conflicts of interest to disclose.

**References**


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