Influence of Osmotic and Oncotic Factors on Gentamicin and Insulin Transport Across the Peritoneal Membrane In Vitro

Glucose or its polymer is usually added to dialysis solution for the development of sufficient ultrafiltration during peritoneal dialysis. The aim of the present study was to determine the influence of glucose and icodextrin on the transport of gentamicin and insulin from the mesothelial to the interstitial side of the peritoneal membrane. Transfer values are expressed as a coefficient of diffusive permeability, \( P \), in centimeters per second. Each of the molecules was tested in 3 series of experiments using rabbit parietal peritoneum, a modified Ussing chamber, and a mathematical model of mass transport. First, transperitoneal transfers of gentamicin (0.040 g/dL) and insulin (0.1 g/dL) were analyzed in control conditions for 120 minutes. Then, transport parameters for gentamicin and insulin were separately determined before (15 – 60 minutes) and after (75 – 120 minutes or 75 – 130 minutes) the application of glucose (1.8 g/dL) or icodextrin (2 g/dL) on the mesothelial side of the peritoneal membrane. Insulin transport was observed to be stable in the control series. Gentamicin transfer was not stable; its passage declined by 52% (\( p < 0.01 \)) in the control series. The mean transfer parameters were 7.41 ± 1.40 cm/s (\( \times 0.0001 \)) over 15 – 30 minutes and 3.21 ± 0.54 cm/s (\( \times 0.0001 \)) over 75 – 130 minutes. Gentamicin transfer declined less in the series with glucose or icodextrin, by 21% (\( p < 0.04 \)) and 30% (\( p < 0.05 \)) respectively, than in the control series. For insulin, the mean \( P \) (± standard error of the mean) was 0.15 ± 0.02 cm/s (\( \times 0.0001 \)) at the first hour of transfer and 0.14 ± 0.02 cm/s (\( \times 0.0001 \)) at the second.

Glucose induced a nonsignificant intensification of insulin transport. Icodextrin increased insulin passage by 107% (\( p < 0.03 \)). Osmotic and oncotic factors (glucose and icodextrin) both stabilize the transfer of gentamicin across the peritoneal membrane in vitro. Glucose polymer intensifies insulin transport from the mesothelial to the interstitial side of the peritoneum. Similar modifications might be observed in vivo during peritoneal dialysis or continuous intraperitoneal administration of insulin, influencing the efficiency of those treatments.

Key words
Peritoneal transport, glucose, icodextrin, gentamicin, insulin

Introduction
Peritoneal dialysis (PD)—an established, well accepted, and adequate renal replacement therapy—is used by 5% – 80% of the dialysis population in various countries (1). 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 (2). In standard PD fluids, concentrated glucose (1.36 – 3.86 g/dL—that is, 15 – 40 times the physiologic concentration) is used to develop sufficient ultrafiltration during exchanges. The glucose polymer called icodextrin can be substituted for the monomer, thereby avoiding some of the hexose-dependent effects (1,3,4).

The special properties of isosmotic 7.5 g/dL icodextrin solution include induction of transcapsillary ultrafiltration by a mechanism resembling colloid osmosis. For example, high molecular weight (MW)
glucose polymer (about 13 – 19 kDa) has been successfully used during peritonitis and can prevent the temporary decline of ultrafiltration that typically occurs because of the increased absorption of glucose induced by inflammation in the peritoneal membrane when glucose-based solution is applied (3). Icodextrin can be used alone in dialysis solutions or in combination with glucose, in 1 or 2 dwells during daily treatment (5).

Peritonitis is a major complication of PD and one of the main reasons that patients are transferred to hemodialysis. To treat peritoneal inflammation, antibiotics are preferentially delivered by the intraperitoneal (IP) route so that maximal concentrations are applied at the site of infection, with a lesser risk of systemic toxicity (6). Notably, icodextrin-containing PD solutions are stable with the various antibiotics commonly used for the treatment of peritonitis—for example, gentamicin (7).

In the past, because of the risk of further renal function decline, gentamicin was not recommended for use in PD patients who retained some residual renal function. A later study showed that short-term use of this aminoglycoside is safe and does not compromise renal function. Recently, gentamicin has been listed as one option for empiric gram-negative antibiotic cover (6,8) in PD-related peritonitis. The transfer dynamics of this aminoglycoside through the peritoneal membrane and the factors that influence its passage are little recognized. Moreover, gentamicin (MW: 478 Da) has been considered a surrogate marker for molecules at the high end of the small-molecule range (middle-molecule solutes weigh between 500 Da and 15000 Da).

In patients on PD and in those not on any dialysis therapy, IP insulin treatment may have advantages, including direct delivery of the drug to the liver and better insulin sensitivity (9). Compared with subcutaneous insulin, continuous IP administration of insulin allows for blood glucose values to normalize more rapidly after a meal, with a more predictable profile (10,11). Much of the IP insulin is absorbed through the portal system, which more closely mimics normal physiologic action, resulting in improved hepatic uptake and lower peripheral levels of plasma insulin (11,12). Many factors probably influence the dynamics of this hormone transfer after IP infusion, but details of the process are little known.

The aim of the present in vitro study was to verify the role of glucose and its polymer in the transfer of gentamicin and insulin through the peritoneal membrane.

Methods
The experiments used fragments of parietal peritoneum from New Zealand male rabbits (Local Ethics Committee for Animal Research in Poznań, Poland, approval no. 47/2009), which were placed into a modified Ussing chamber system. The active surface area of the membrane was 1.1 cm². The chamber was connected through a peristaltic pump to the fluid reservoir (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), circulating 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 50 mL/L CO₂ and 950 mL/L O₂. The whole system was placed in a thermostatic box at 37°C (13,14).

We determined the diffusion rate of gentamicin [initial concentration gradient: 0.04 g/dL; MW: 478 Da (Polfa Tarchomin, Warsaw, Poland)] and insulin [initial concentration gradient: 0.1 g/dL; MW: 6 kDa (MP Biomedicals, Solon, OH, USA)] from the mesothelial to the interstitial side of peritoneal membrane. For each molecule, 3 separate series of transfer analyses were carried out:

- In control conditions without glucose or icodextrin (120 minutes)
- Before (15 – 60 minutes) and after (75 – 120 minutes) the application of glucose [1.8 g/dL; MW: 180 Da (Poch, Gliwice, Poland)] on the mesothelial side of the membrane
- Before (15 – 60 minutes) and after (75 – 130 minutes for gentamicin or 75 – 120 minutes for insulin) the application of icodextrin [2 g/dL; average MW: 15 kDa (ML Laboratories, Liverpool, England)] on the mesothelial side of the membrane

The osmolality of the solutions amounted 400 mOsm/kg H₂O for the monomer and 300 mOsm/kg H₂O for the polymer. Sampling of the medium was carried out at regular 15-minute intervals.

A mathematical model of mass transport was
used to estimate a diffusive permeability coefficient, $P$ (scaled to the surface area of the investigated membrane), for the study specimens. 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 the mean ($\pm$ 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 (13).

The statistical analyses used the Wilcoxon test for paired data (Statistica 8: StatSoft, Tulsa, OK, U.S.A.). A Shapiro–Wilks test was applied to evaluate the data distribution. Values of $p$ less than 0.05 were considered statistically significant.

**Results**
In control conditions, the dynamics of gentamicin were not stable. Transport of this aminoglycoside declined by 52% ($p < 0.01$) over the observation period. The mean $P$ was $7.41 \pm 1.40$ cm/s ($\times 0.0001$) in the first hour of the experiment and $3.21 \pm 0.54$ cm/s ($\times 0.0001$) in the second hour (Figure 1). Gentamicin transfer declined less in the series with glucose or icodextrin, by 21% ($p < 0.04$) and 30% ($p < 0.05$) respectively, than in the control series [Figure 2(A)].

During the 120 minutes of the control experiment, the rate of insulin transport from the interstitial to the mesothelial side of membrane remained constant (Figure 1). For insulin, the mean $P$ was $0.15 \pm 0.02$ cm/s ($\times 0.0001$) at 15 – 60 minutes and $0.14 \pm 0.02$ cm/s ($\times 0.0001$) at 75 – 120 minutes. Glucose induced a nonsignificant 31% intensification of insulin transport. Icodextrin increased passage of this hormone by 107% [$p < 0.03$; Figure 2(B)].

**Discussion**
Pharmacotherapy by the IP route is an important aspect of PD and has been used for various drugs, most particularly antibiotics (for example, gentamicin) and hormones (for example, insulin) to achieve local and systemic effects (6,8,11,12). As mentioned earlier, studies of transperitoneal transfer of gentamicin and insulin are limited, being related

![Figure 1](image1.png)

**Figure 1** Changes of diffusive permeability ($P$) ± standard error of the mean of rabbit peritoneum for gentamicin (0.04 g/dL) and for insulin (0.1 g/dL) expressed as a percentage of the control value obtained in the first hour of the experiment.

![Figure 2](image2.png)

**Figure 2** Changes of diffusive permeability ($P$) ± standard error of the mean of rabbit peritoneum for (A) gentamicin (0.04 g/dL) and (B) insulin (0.1 g/dL) caused by introduction of glucose (1.8 g/dL) or icodextrin (2 g/dL) into the research system, expressed as a percentage of the control value obtained before the change in experimental conditions.
mainly to plasma kinetics and metabolism. Moreover, few data are available about factors that can change their transport (15).

We observed a decline in gentamicin transfer from the mesothelial to the interstitial side of membrane during the 120 minutes of the control experiments. Similarly, clinical study of this antibiotic showed a rapid rise in blood levels in the first hour after IP delivery, with a slow drop over the next 5 hours. Those findings appear to be the result of a strong positive electric charge of this drug, specific membrane effects, and a subsequent self-limitation of gentamicin transfer. During PD, a decline in the dialysate-to-plasma (D/P) ratio and clearance of urea and creatinine, without a change in ultrafiltration, has been observed with gentamicin administration (16). Moreover, in previous in vitro studies, gentamicin reduced the permeability of the peritoneal membrane to urea and albumin (17). In our series, gentamicin transport declined less with the application of glucose (1.8 g/dL) or icodextrin (2 g/dL) into the research system than it did in control conditions (without osmotic or oncotic factors).

In vitro studies showed that glucose intensifies the peritoneal passage of creatinine, but that icodextrin does not change the transfer of this solute through peritoneum (18). During PD with glucose (3.86 g/dL) or its polymer (7.5 g/dL), transperitoneal transfer of small molecular weight solutes such urea and creatinine are usually similar. When lower concentrations of dextrose (2.5 g/dL) are applied, small-solute clearance and ultrafiltration have been shown to be lower (19), although another long-term study showed that, compared with 4.25 g/dL dextrose, icodextrin (2 g/dL) increased peritoneal clearance of those compounds (20). Those diverse results are probably a result of the various models and concentrations of solutes used, and of the effects of different membrane statuses and of various compounds present in dialysis fluid.

Transport of insulin from the interstitial to the mesothelial side of the peritoneal membrane was stable over 120 minutes in control conditions in vitro. Clinical studies demonstrated high inter-patient variability in insulin kinetics and in parameters of the glucose–insulin system in PD patients with diabetes (9,10,15,21). Individual peaks in plasma insulin concentration occurred after 30 and 60 minutes of a PD dwell using solution with a glucose concentration 3.86 g/dL, the average increases over initial values being 52% and 168% respectively (10). Moreover, the mean absorption of insulin depends on dwell time: 21% is absorbed in a 2-hour dwell, and 46% in an 8-hour dwell (9). In patients with lower blood glucose concentrations, peritoneal insulin absorption at higher percentages has been observed. The transperitoneal insulin concentration gradient and the absorptive surface area are perhaps the major factors determining hormone absorption. Higher insulin absorption was observed with smaller infused volumes of dialysate (10).

The relationship between insulin absorption percentage and peritoneal membrane transport is controversial (10,21). Pantsulaia et al. observed a positive correlation between insulin absorption and peritoneal equilibration test results for creatinine (10), but Fine et al. found no relationship between the amount of insulin absorbed and the D/P creatinine (21). In our analysis, glucose slightly intensified insulin transport. The observed change was not statistically significant, probably because of inter-animal variability, also observed in human studies. By contrast, the transfer of icodextrin increased by 107% in vitro. In our previous studies, glucose polymer intensified albumin transfer through the peritoneum (22). Moreover, glucose intensified icodextrin passage through the peritoneal membrane in vitro (23). Use of glucose polymer instead of its monomer during PD resulted in a reduction, by a factor of 2.5, of the daily requirement for insulin (in units) in diabetic patients, mainly because of less glucose absorption (4). Clinical studies with fluid containing icodextrin (7.5 g/dL) compared with fluid containing glucose (3.86 g/dL) have observed an increase in the transperitoneal transport of β2-microglobulin, leptin, cystatin C, and another small protein (24). A similar mechanism probably plays a role in the transperitoneal transfer of insulin, a middle molecule (MW: 6 kDa).

These findings of higher peptide transport with the use of icodextrin support the hypothesis that glucose polymer acts through the small pores and increases solvent drag. That phenomenon is likely caused by increased convective transport of solutes, including peptides and small proteins, through the small pores. The radii of β2-microglobulin (16 Å; MW: 12 kDa) and cystatin C (15 Å; MW: 13 kDa) allow passage through the small pores. By contrast, analyses of albumin (36 Å; MW: 67 kDa) and α2-macroglobulin (90 Å; MW: 720 kDa) clearances found no transport...
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differences whether icodextrin or hypertonic glucose solution was used (3,24).

Conclusions
Given the present data, we can conclude that in vitro peritoneal transport of gentamicin decreases by 52% over 120 minutes, but that the dynamics of insulin transfer are stable over 2 hours. Glucose and icodextrin stabilize the passage of gentamicin through the peritoneum. Glucose polymer intensifies peritoneal absorption of insulin. The causes of these effects are not recognized, but the results, which can be observed in vivo during PD or continuous IP administration of insulin, can be important to the efficiency of those treatments.

References
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