

## Factors Affecting Icodextrin Peritoneal Transport *In Vitro*

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We undertook *in vitro* experiments to examine the importance of mesothelium and interstitium in icodextrin (7.5 g/dL) transport and the change in that transport caused by gentamicin and methylglyoxal. Rabbit peritoneum, a modified Ussing chamber, and a mathematical model of mass transport were used. Transfer from the interstitial to mesothelial side of the membrane ( $I \rightarrow M$ ) and in the opposite direction ( $M \rightarrow I$ ), expressed as a diffusive permeability coefficient  $P$ , was determined in control series, after chemical modification of the peritoneum by sodium deoxycholate, and after introduction of gentamicin and methylglyoxal. We also investigated the thickness of native tissue 75 minutes into the study and after use of sodium deoxycholate.

In the control series, icodextrin  $I \rightarrow M$  transport increased by 50%, but  $M \rightarrow I$  transport remained stable [15 – 60 min vs. 75 – 120 min:  $I \rightarrow M P$ ,  $0.32 \pm 0.04 \times 10^{-4} \text{ cm} \cdot \text{s}^{-1}$  (standard error of the mean);  $M \rightarrow I P$ ,  $0.19 \pm 0.03 \times 10^{-4} \text{ cm} \cdot \text{s}^{-1}$ ]. After application of sodium deoxycholate,  $I \rightarrow M$  transport was observed to increase by 21% and  $M \rightarrow I$  by 192% as compared with the 2nd hour of the control series. Gentamicin caused a rise of  $M \rightarrow I$  transport by 21% without a change of  $I \rightarrow M$ . We observed no difference in  $p$  values ( $I \rightarrow M$  and  $M \rightarrow I$ ) after application of methylglyoxal. Mean thickness before and 75 minutes into the study was  $4.96 \pm 0.28 \mu\text{m}$  for mesothelium and  $62.09 \pm 2.40 \mu\text{m}$  for the whole peritoneum. Sodium deoxycholate reduced the mesothelium thickness by 20% and increased the peritoneum thickness by 37%.

The present study confirms that, *in vitro*, icodextrin  $I \rightarrow M$  peritoneal transport changes with time, but  $M \rightarrow I$  is constant. Asymmetry of glucose polymer diffusion is observed:  $I \rightarrow M$  predominates over  $M \rightarrow I$ . Chemical modification of the peritoneum by sodium deoxycholate ( $I \rightarrow M$  and  $M \rightarrow I$  directions) and by gentamicin ( $M \rightarrow I$  direction only), but not by methylglyoxal, intensifies icodextrin transport.

*Sodium deoxycholate causes exfoliation of the mesothelium and looseness of the interstitium.*

### Key words

Peritoneal transport, icodextrin, sodium deoxycholate, gentamicin, methylglyoxal

### Introduction

The removal of excess water from the body of patients with renal disease is a priority of peritoneal dialysis. Water removal is accomplished by the introduction of hypertonic glucose solution into the abdominal space. Recently, isotonic fluid with a glucose polymer called icodextrin has been used for this purpose. Icodextrin is able to induce sustained ultrafiltration equivalent to that achieved with a glucose exchange (1,2).

The routes and mechanisms of glucose polymer peritoneal transport are not well defined (2). Intra-peritoneally introduced icodextrin (7.5 g/dL) is absorbed slowly (about 40% of the administered dose during a 12-hour dwell), probably mainly by the lymphatic pathway. Diffusion is limited, but not excluded. In blood, icodextrin is hydrolyzed by  $\alpha$ -amylase to oligosaccharides. These solutes may be metabolized to glucose by tissue maltases and excreted into the urine or eliminated by peritoneal dialysis (1).

The aim of the present study was to examine the dynamics of bidirectional diffusive transfer of icodextrin across the intact and chemically modified (by gentamicin and methylglyoxal) peritoneal membrane. Morphometric analyses of the thickness of the peritoneal tissues in these circumstances were also undertaken.

### Material and methods

Fragments of peritoneum were isolated from the abdominal wall of New Zealand rabbits. These tissues were placed into a modified Ussing chamber connected to a reservoir of Hanks solution and a peristaltic pump in a thermostatic box at 37°C (2–4). The procedure was approved by the Local Ethics Committee for Animal Research in Poznań, Poland.

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Icodextrin (7.5 g/dL; ML Laboratories, Liverpool, U.K.) transport directed from the interstitial to mesothelial side of the peritoneum (I→M), and in the opposite direction (M→I), was evaluated

- in control conditions (15 – 120 minutes; “control series”).
- before (15 – 75 minutes) and after (90 – 150 minutes) chemical modification of the peritoneum by sodium deoxycholate (104 mg/dL; POCH, Gliwice, Poland).
- before (15 – 60 minutes) and after (75 – 120 minutes) application of gentamicin (2 mg/dL; Polfa, Tarchomin, Poland).
- before (15 – 60 minutes) and after (75 – 120 minutes) application of methylglyoxal (1 mg/dL; Sigma–Aldrich, St. Louis, MO, U.S.A.).

Icodextrin was determined after hydrolysis to glucose by amyloglucosidase, and glucose was measured using glucose oxidase (Cormay, Lublin, Poland). The results were expressed as a diffusive permeability coefficient  $P \pm$  standard error of the mean [SEM (2–4)]. The data from the series with added sodium deoxycholate, gentamicin, and methylglyoxal were compared with the results obtained in the control series—specifically, data from the 2nd hour (75 – 120 min) of the control series.

In addition, the thickness  $T \pm$  SEM of the mesothelium and the whole peritoneum were evaluated at three points:

- Immediately after isolation from the rabbit (native tissue)
- 75 Minutes into the study with the Hanks solution alone
- After application of sodium deoxycholate

Tissues were prepared using standard histology procedures, and  $T$  was estimated using an Olympus CX 40 microscope (Olympus, Center Valley, PA, U.S.A.) and the ImageJ slide analysis software from the U.S. National Institutes of Health.

In the statistical analyses, we applied the Wilcoxon test, Mann–Whitney test, and Student  $t$ -test as appropriate. A value of  $p \leq 0.05$  was considered statistically significant.

## Results

In the control series, at 120 minutes of the experiment, the rate of icodextrin I→M transport was higher than M→I transport, and  $P$  values were  $0.32 \pm 0.04 \times 10^{-4} \text{ cm} \cdot \text{s}^{-1}$  and  $0.19 \pm 0.03 \times 10^{-4} \text{ cm} \cdot \text{s}^{-1}$  respectively. Dynamics of M→I diffusion remained constant, but I→M increased by 50% over time (15 – 60 min vs. 75 – 120 min) (2).

In the series with sodium deoxycholate, icodextrin transfer increased by 21% (I→M) and 192% (M→I) as compared with the control series (hour 2). Moreover, gentamicin enhanced  $P$  by 21% for M→I glucose polymer transport, but did not change transport in the opposite direction. After application of methylglyoxal, no modifications were observed in the bidirectional icodextrin diffusion as compared with diffusion in the control series (Figure 1).

Mean values ( $\pm$  SEM) of  $T$  for the mesothelial layer were  $5.05 \pm 0.23 \mu\text{m}$  at the beginning of the study and  $4.87 \pm 0.34 \mu\text{m}$  at 75 minutes. For the peritoneal membrane (mesothelium plus interstitium),  $T$  was  $60.35 \pm 2.53 \mu\text{m}$  and  $63.83 \pm 2.27 \mu\text{m}$  respectively. Sodium deoxycholate caused a 20% reduction in the  $T$  of the mesothelium, but a 37% increase for the whole peritoneum as compared with values at 75 minutes into the experiment (Figure 2).

## Discussion

Clinical studies show that intraperitoneally applied icodextrin is absorbed across the peritoneal membrane at a low but constant rate (1). In our *in vitro* studies, transport of glucose polymer directed from the mesothelial to interstitial side of the peritoneum was also stable. However icodextrin diffusion in the opposite direction increased with time. Moreover, we observed asymmetrical transfer: I→M predominated over M→I. The reasons for this phenomenon are unknown (2). It may be connected with the partial degradation of icodextrin to oligosaccharides, with a resulting increase in fluid osmolality and faster glucose polymer transport. Icodextrin breakdown related to high amylase activity has been observed in rodents. In humans, amylase activity in peritoneal effluent was found to be undetectable, and the intraperitoneal metabolism of icodextrin was very low (1,2). Furthermore, structural and functional peritoneal modifications induced by glucose polymer cannot be excluded in the observed asymmetrical diffusion.

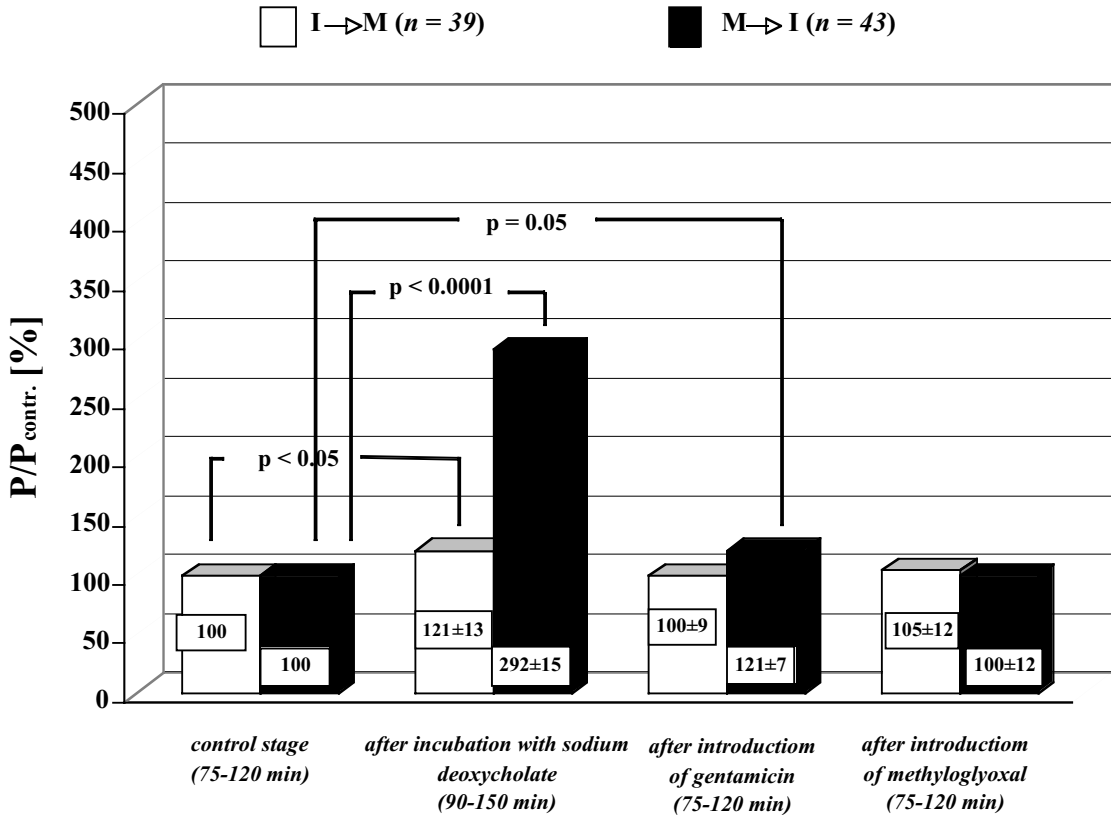


FIGURE 1 Diffusive permeability  $P$  of rabbit peritoneum [ $\pm$  standard error of the mean (SEM)] for icodextrin (7.5 g/dL) and after application of sodium deoxycholate (104 mg/dL), gentamicin (2 mg/dL), and methylglyoxal (1 mg/dL), expressed as a percentage of the values obtained in the control series (specifically in the 2nd hour). I = interstitial side of the peritoneum; M = mesothelial side of the peritoneum.

When mesothelial cells in mice were exposed to icodextrin, they displayed reduced density and cell viability, but increased cell size and nucleus:cytoplasm index (5). These morphologic changes caused disturbances in peritoneal transport function. A rise in protein excretion after single or long-term intraperitoneal use of glucose polymer was observed in rats (6). Previous *in vitro* results with icodextrin showed modification of uric acid and albumin transfer across rabbit peritoneum (3).

The capillary endothelium, interstitium, mesothelium, and stagnant fluid layer restrict icodextrin peritoneal transport (1,2). In the present study, we considered the importance of the mesothelial and interstitial tissues in that process. Our morphometric

analyses showed that the mean  $T$  of rabbit parietal mesothelium plus interstitium was about 60  $\mu\text{m}$ . A similar result was obtained for human parietal peritoneum, with the  $T$  of that membrane amounting to 30–70  $\mu\text{m}$  (7).

The peritoneal pathway for solutes can be modified by uremia, long-term peritoneal dialysis, or peritonitis. In our analyses, a model of chemically modified peritoneum was used as an equivalent of the membrane during peritonitis. Sodium deoxycholate, which is known to denude the pleural mesothelium and epithelial cells of renal tubules (8), changed the peritoneum. Sodium deoxycholate is also known to induce apoptosis and necrosis of the mesothelium, lipid peroxidation, generation of free radicals, and

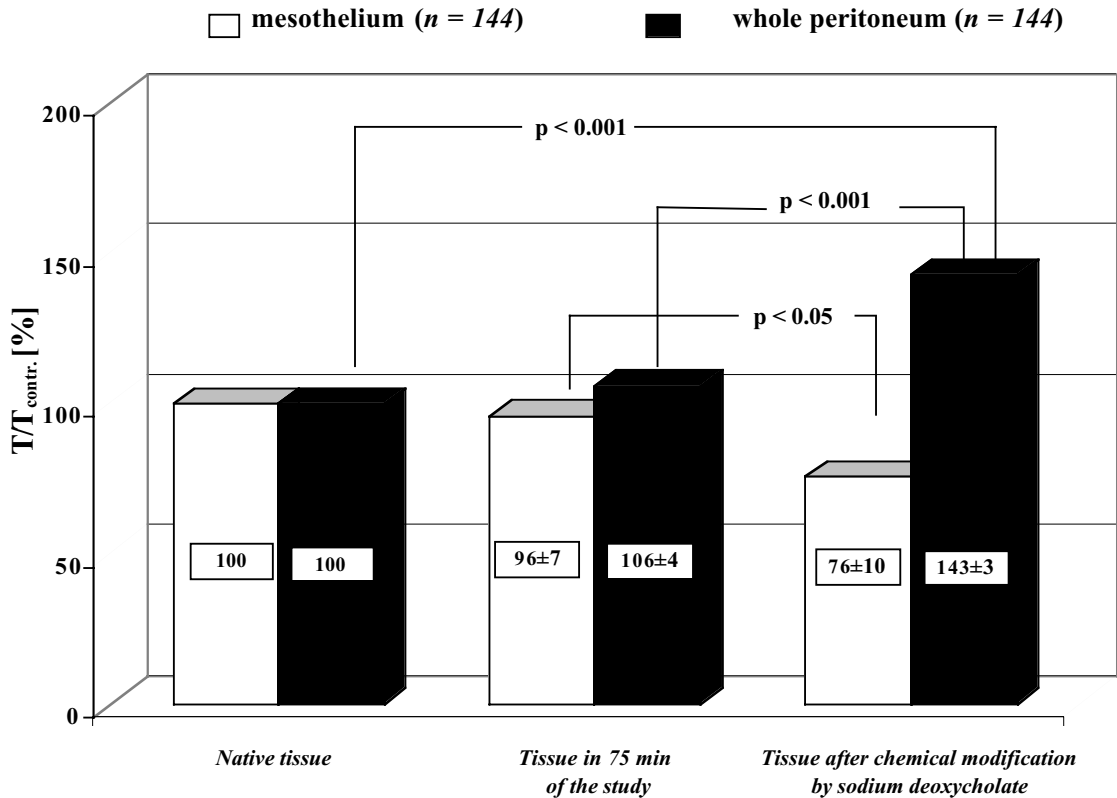


FIGURE 2 Thickness  $T$  [ $\pm$  standard error of the mean (SEM)] of the mesothelium and the whole peritoneum (mesothelium plus interstitium), at 75 minutes into the study and after chemical modification by sodium deoxycholate (104 mg/dL), expressed as a percentage of the control values obtained for the native tissue (after isolation from the rabbit).

histamine liberation from peritoneal mast cells (9). Our morphometric evaluation showed that sodium deoxycholate damaged the mesothelial cells and loosened the adjacent connective tissue. We observed a decrease in mesothelium thickness, but an increase in the interstitium. Furthermore, after application of sodium deoxycholate, we noted a rise in bidirectional icodextrin transport (as compared with the control series), but with a greater rise in the M→I direction than in I→M. Few data about the effect of peritonitis on icodextrin transfer are available. An increase in glucose polymer degradation and dialysate osmolality were observed in rats during peritoneal inflammation (10).

Intraperitoneal delivery of antibiotics is not rare; this route is often used in cases of peritonitis (4,11).

Despite its well documented oto- and nephrotoxicity, gentamicin is used in this way to fight gram-negative bacterial infections. *In vivo*, gentamicin was observed to decrease the dialysate-to-plasma ratios and clearance of urea and creatinine, but not to alter ultrafiltration and renal elimination of sodium and potassium (11).

In the present study, we analyzed the direct influence of gentamicin on icodextrin transport. As compared with the control series, introduction of the antibiotic into the experimental system was observed to be followed by an increase in M→I glucose polymer transport. Diffusion in the opposite direction did not change. In previous *in vitro* studies, gentamicin reduced bidirectional transport of urea and albumin and modified the diffusion dynamics of uric acid, but did not affect inulin transfer (4). The mechanisms of

this action by gentamicin on peritoneal permeability is unknown. It appears to result from a specific membrane effect and the strong positive electric charge of gentamicin (4,11).

Methylglyoxal is one of the glucose degradation products (GDPs) generated during heat sterilization of conventional fluids for peritoneal dialysis (12–14). Its cytotoxicity is well documented, but few data about its influence on peritoneal transport are available (12,15). *In vivo* elimination of methylglyoxal and other GDPs from acidic dialysate did not change ultrafiltration or the mass transfer area coefficient for glucose and  $^{51}\text{Cr}$ -EDTA, but these parameters decreased in neutral solution (12). Our analyses showed that methylglyoxal did not change bidirectional transport of icodextrin across the peritoneum as compared with transport in the control series. However, in earlier *in vitro* studies, an increase in glucose transfer and a decrease in creatinine diffusion were observed (15).

It has been suggested that the action of intraperitoneally applied methylglyoxal is multidirectional. It modifies the morphology and function of peritoneal cells and other tissues (12–14,16). A swelling of pancreatic  $\beta$ -cells and a reduction in submesothelial peritoneal tissue thickness were observed in rats after application of methylglyoxal (12,16). These morphologic changes are probably related to the protein glycation induced by methylglyoxal. Intense immunostaining of anti-advanced glycation end-product antibodies was noted in methylglyoxal-treated rats; such staining was suppressed in animals treated with sodium sulfate (13). Methylglyoxal also caused functional disturbance in tissues. It intensified the production of vascular endothelial growth factor, inhibited the release of interleukin 6 and fibronectin from peritoneal cells, and decreased the mitochondrial permeability transition in liver (13,14,17).

### Conclusions

Peritoneal transport of icodextrin *in vitro* shows asymmetry and change over time:  $\text{I} \rightarrow \text{M}$  is higher than  $\text{M} \rightarrow \text{I}$ ; and  $\text{I} \rightarrow \text{M}$ , but not  $\text{M} \rightarrow \text{I}$ , decreases by 50% over 120 minutes. These modifications may be related to the partial degradation of glucose polymer and to structural and functional peritoneal membrane disturbances induced by icodextrin.

Gentamicin, but not methylglyoxal, slightly enhanced unidirectional ( $\text{M} \rightarrow \text{I}$ ) icodextrin diffusion *in vitro*.

Sodium deoxycholate caused injury to mesothelial layer, looseness in the adjacent connective tissue, and augmentation of bidirectional icodextrin transfer, especially in the  $\text{M} \rightarrow \text{I}$  direction.

These results may be important for the efficiency of peritoneal dialysis and intraperitoneal pharmacotherapy.

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