EVALUATION OF THE PARTICIPATION OF PARIETAL PERITONEUM IN DIALYSIS: PHYSIOLOGICAL, MORPHOLOGICAL AND PHARMACOLOGICAL DATA

J Knapowski, E Feder, M Simon, M Zabel

University Medical School, Poznań, Poland

Summary

Bidirectional fluxes of Na+, K+ and Ca++ were estimated under in vitro conditions through isolated patches of rat peritoneal membranes (parietal and visceral). Morphological (TEM) and functional (changes in fluxes) data are presented to show that mesothelium lining the parietal peritoneum forms a barrier that limits diffusion of ions across this membrane. In addition, it was found that Furosemide and nitroprusside change the permeability of the mesothelium for sodium or potassium, respectively.

Introduction

The diaphragm and anterior abdominal wall are covered on their caval side by mesothelial peritoneal membrane [1,2] with a surface area in man of the order 800 – 1100 and 800 – 900cm², respectively. This is not a very great area in comparison with the figure of 1.5 – 2.0m² estimated for the total peritoneal area [3,4], but it does not presuppose a negligible role of this membrane during the dialysis process. Morphological examination of this membrane revealed that mesothelial cells are organised as an epithelial-like structure, and this suggests that simple intercellular diffusion is not the only mode of transfer of substances across this barrier but suggests the existence of transcellular movement.

As the latter form of biological transport plays a regulatory role in several tissues, being simultaneously influenced by various factors, both endogenous (e.g. hormones) and exogenous (e.g. drugs), one can expect the parietal mesothelium to act similarly in the peritoneal cavity.

To check the above suggestions, as no data regarding the physiological function of the parietal peritoneum exist in the relevant literature, a series of experiments were performed in vitro on patches of this membrane.
Materials and Methods

The technique has been previously described in detail [5] and in brief it is as follows: small pieces of peritoneal membrane were taken (as carefully as possible) from the abdomen of anaesthetised Wistar rats and mounted into a double Ussing-type chamber which made it possible to measure bidirectional radioisotope* fluxes across the membrane, as free admittance to medium† bathing both sides of the membrane was allowed. Figure 1 explains the manner in which the membranes were taken up, and the experiments performed.

Each experiment lasted 5–6 hours, and several samples of medium from both sides were taken for measurement of radioactivity+ at consecutive 1/2 hr intervals.

In some of the experiments on parietal peritoneal mesothelial cells were intentionally desquamated. This was accomplished mechanically by a gentle scraping with the razor just before mounting of the membrane, or chemically by addition of Na-deoxycholate (5mM) to the medium bathing the mesothelial side at the beginning of the experiment: both procedures were followed by rinsing with fresh medium. A series of systematic morphological searches using transmission electron microscopy (JAM 100-C) was carried out. Specimens of the membranes were taken immediately from the body or from the chamber after the whole experiment (5 hrs) was completed; the latter group comprised membranes with mesothelium removed mechanically or by deoxycholate and intact membranes as well. Comparison of the two latter types of membrane is shown in electron-micrographs (Figure 2a/b).

During the course of 53 double experiments in which bidirectional fluxes of sodium and/or potassium were measured through patches of both types of parietal peritoneum (with mesothelium retained), Furosemide (Polfa, Poland) or sodium nitroprusside were added (usually after 120 min) in final conc. of 1 x 10⁻⁴ or 5 x 10⁻⁴ M, and 1.5 x 10⁻⁵ or 1 x 10⁻³ M, respectively, to the medium bathing the mesothelial side of the membrane.

Results

Electron microscopic examination showed a normal appearance of mesothelial cells covering the parietal peritoneum despite the membranes being maintained 5 hours under in vitro conditions (Figure 2a), provided that the procedure of their separation from the body was sufficiently gentle. On the other hand the series revealed that both methods of removal of mesothelium were effective while the basement membrane remained intact (Figure 2b).

Comparison of these results with the flux measurements leads to the firm

*²Na and §⁵Ca from Radiochemical Centre, Amersham, England; §⁶Rb (as label of potassium) from Radioisotope Unit, Swierk, Poland.
†Hanks solution; pH 7.4 and oxygenation kept constant by intensive bubbling with O₂ +CO₂ gas mixture.
+tin scintillation counters: Ultra-Gamma 1280 LKB, Wallac or LS-100C, Beckman.
Figure 1. Procedure for separation of two types of parietal peritoneal membranes for measurements of bidirectional fluxes of ions in vitro; mesentery was also taken for similar flux measurements but of indefinable direction.
Figure 2a. A survey electron micrograph of a mesothelial cell covering the aaw-peritoneum maintained 5 hours under in vitro conditions. No sign of damage found in comparison with cells of membranes taken immediately from the body (x 40,000 – reduced for publication)

Figure 2b. An electron micrograph of the aaw-peritoneum treated with deoxycholate at the beginning of the experiment: mesothelium completely removed whereas the basement membrane retained (x 40,000 – reduced for publication)
Figure 3. Average values (±SEM) of ion fluxes (nmoles/sec·cm²) across both types of parietal peritoneum (with mesothelium retained or after its removal) in comparison with relevant fluxes across mesentery. All experiments carried out under the same conditions.
Figure 4. Effect of nitroprusside (1.5x10^{-5} and 1x10^{-4} M) on bidirectional flux of potassium across the diaphragm in vitro (pleural mesothelium removed before the experiment whereas the peritoneal one retained throughout); only unidirectional flux (vascular-to-mesothelial) was increased, and this may lead to increase of potassium peritoneal clearance during dialysis (see the diagram to right of Figure)
Figure 5. Effect of furosemide (1 x 10^{-4} M) on bidirectional flux of sodium across the diaphragm peritoneum in vitro; mesothelial-to-vascular flux but not in the other direction was affected; a proposition as regards the final effect of this action during dialysis is shown to the right of the Figure.
conclusion that the mesothelial monolayer forms a barrier that limits free diffusion of ions across the parietal peritoneum, and that it must play a role in the transporting functions of this membrane. Average values of bidirectional fluxes of sodium, potassium and calcium across both types of parietal peritoneum (diaphragm and anterior abdominal wall (aaw), either with or without mesothelium) that were found between 150 – 240 min of the course of the experiments, are presented in Figure 3. Values for the relevant fluxes across the mesentery are also inserted into the diagram for comparison. Differences between all these groups may be summarised as follows:

1 All ions diffused most intensively through the mesentery, whereas their transfer across the parietal peritoneum - especially the aaw-peritoneum - was much less.

2 Diffusion of sodium was greater than potassium and even more than calcium.

3 Removal of mesothelium from the parietal peritoneum membrane causes a significant increase of all fluxes measured (the only exception was flux of calcium through aaw-peritoneum although the results in this group are provisional.

4 Transfer of sodium and potassium across the parietal peritoneum with intact mesothelium showed unequal intensity in the two directions: usually the flux from mesothelial to muscular side (hereafter assumed as 'vascular space') predominated, except sodium transfer across the aaw-peritoneum where a variable situation was observed.

Discussion

It remains an open question how far this elaborate model permits one to make suggestions regarding the phenomena of solute transport that take place in situ. In fact, the model simplifies the problem of transport pathways across the whole membrane; for example the role of capillary endothelium is completely ignored. On the other hand, the method allows one to investigate the phenomena that occur at the level of mesothelial cells.

Addition of furosemide or nitroprusside to medium bathing the parietal peritoneal membranes induced changes in unidirectional fluxes of sodium or potassium, respectively. Despite the small number of experiments completed so far, some of these effects were statistically significant. Figures 4 and 5 illustrate the results of two series of experiments where these effects were particularly clear, showing that in each only one component of ion transport was modified by the drug. Based upon the assumption that peritoneal ion transport in man shows the same characteristics, and that these drugs act there similarly, a calculation was performed (Figures 4 and 5) to indicate a mechanism by which these drugs supposedly augment the peritoneal excretion of sodium or potassium during dialysis.

From these results it seems justifiable to postulate that although the exchange of solutes through parietal peritoneum is presumably small, the changeable permeability of its mesothelium can modify peritoneal excretion during the dialysis process.
Acknowledgments

This work was supported by a grant No. 05-084-N from the Coordinating Commission for Polish-American Scientific Collaboration, and morphological examinations were supported by a grant No. 623/VI from the Polish Academy of Sciences.

The authors wish to thank Dr Benjamin T Burton, Chief of the National Artificial Kidney Program, NIH - Bethesda, for having a constant interest in this work, valuable suggestions made during frequent discussions on the results obtained, as well as for affording possibilities for one of us (J.K.) to make personal contacts with many nephrological and physiological centres in the USA.

References

1 Leak, LV and K Rahil (1978) Amer. J. Anat., 151, 557
2 Baradi, AF and Rao, SN (1976) Tissue and Cell, 8, 159
4 Henderson, LW (1973) Kid. Int., 3, 409

Open Discussion

BOEN (Chairman) Did you also measure the flux of other substances than potassium with nitroprusside?

Knapowski Until now we observed only the effect on the potassium flux.

BOEN Miller showed by in vivo experiments that nitroprusside causes vasodilatation and the increased appearance so far has been attributed to the higher vascular permeability of the peritoneum, but there are no experiments on the in vivo influence of nitroprusside outside the vascular bed. Is Dr Nolph in the audience?

Nolph (Columbia) Nitroprusside does cause changes in vascular permeability and numbers of capillaries perfused, when applied topically to the intact peritoneum in vivo. Such vascular changes probably contribute to increased clearances and protein losses during peritoneal dialysis with addition of nitroprusside to peritoneal dialysis solution. We have now heard that nitroprusside may alter mesothelial permeability in the isolated peritoneum in vitro. Perhaps the clinical effects of intra-peritoneal nitroprusside are not entirely explained by its effects on the microvasculature.

Knapowski These results do not exclude the other mechanisms of action of nitroprusside. Maybe there is the action on vasculature and on the other hand the action on mesothelium.

Walls (Leicester) Is there any evidence that the peritoneal membrane has active ionic transport? If so, have you attempted to inhibit the Na-K pump with
substances such as ouabaine as you have only measured ionic fluxes?

KNAPOWSKI We do not presuppose that there is active transport although our results suggest it; nevertheless some additional experiments should be done to prove whether there is active transport. Presumably there is diffusion in one direction and diffusion in another, and maybe there is an extra mechanism such as a chemical pump which supports the diffusion in one direction; perhaps it is a kind of facilitated diffusion pump, or something like that. We cannot say exactly whether it is active transport or not, but it seems to be very likely.