

## FIRST HISTOLOGICAL FINDINGS IN RABBIT MODEL OF PERITONEAL DIALYSIS

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(Received 5<sup>th</sup> March 2010)

*Investigating peritoneal membrane alterations caused by peritoneal dialysis fluid during peritoneal dialysis in humans is still intriguing. That is why the study was aimed to provide rabbit peritoneal tissue samples suitable for histological analyses using a modified non-uremic infusion model of peritoneal dialysis on rabbit. A double-lumen central venous catheter, surgically placed in the peritoneal cavity of adult Chinchilla rabbit, was used for daily instillations of peritoneal dialysate. Peritoneal tissue samples were obtained during the catheter placement and removal and analysed by light and transmission electron microscopy. The results of histological examinations showed that this modified non-uremic rabbit model of peritoneal dialysis is suitable for obtaining peritoneal tissue samples for light and transmission electron microscopy examination, and can be used to analyze the effects of different dialysis solutions on rabbit peritoneal membrane.*

*Key words: experimental model, histological study, peritoneal dialysis*

### INTRODUCTION

Therapeutic options for end stage kidney disease patients remain to be transplantation (still implemented in the minority of individuals) and blood purification procedures – hemodialysis and peritoneal dialysis. Peritoneal dialysis is a well established dialysis modality, convenient for many patients due to the possibility of individual at home performance (Trbojević *et al.*, 2001).

The dialysis process takes place through the peritoneal membrane, a complex structure consisting of blood vessels endothelial cells, peritoneal lamina propria, basement membrane and mesothelial layer (Michailovaa *et al.*, 2005). The dialysis solution is instilled in the peritoneal cavity and an exchange of substances based on diffusion and osmosis takes place (Witowski and Jorres, 2009), so that uremic waste products from blood and excess body fluid are dialysed in the solution. In order to enable excess water removal into the body, the

dialysate contains unphysiological levels of glucose as the osmotic agent. This constant exposure to hyperosmolar and low pH dialysis solution, with high glucose concentration causes additional injury to the peritoneal mesothelium, already damaged by the uremic internal milieu (Obradović *et al.*, 2000; Stojimirović *et al.*, 2000; Trpinac *et al.*, 2002).

It is still a challenge to investigate the influence of the dialysate on human peritoneal membrane due to ethical and technical limitations. Therefore, *in vivo* animal models are developed to provide important informations on structural changes in the peritoneum, on peritoneal transport pathophysiology and local defense mechanisms. There is no consensus on the ideal experimental model so far (Mortier *et al.*, 2005).

This study was aimed to provide rabbit peritoneal tissue samples suitable for histological analyses with light and transmission electron microscopy examination using a modified non-uremic infusion model of peritoneal dialysis on rabbit, which would be easy to perform, reproducible, and inexpensive.

#### MATERIAL AND METHODS

##### *Experimental animal*

An adult healthy Chinchilla rabbit, weighing 2700 g at the beginning of the study, was followed up. Before catheter insertion, the rabbit was acclimatized for five days in an individual cage, allowed free access to food (standard rabbit pellets, Veterinary Institute, Serbia) and water. The food and water were stopped the day before the surgery and allowed the following day. A diary, including data concerning body weight, body temperature, food intake, diuresis, defecation, antibiotics administration, other therapy and interventions if necessary (wound toilette, catheter suturing etc.) was kept.

##### *Surgical procedure*

A double-lumen central venous catheter (Arrow International Inc. USA Product No.CV-17702-E) was used as a peritoneal catheter in order to perform dialysate instillations.

Surgical procedures for catheter placement was a modified version of the procedure described in the literature (Schambye *et al.*, 1992; Zweers *et al.*, 1999). The animal was anesthetized following existing protocols, with Thiopental BP 1G (Rotexmedica, Germany), 0.5 mg/kg body mass, the ear vein shaved, and the operative field was prepared in a standard way. A longitudinal incision, 3 to 4 cm long, located 2-3 cm laterally from the left costal arch edge and 4-5 cm from the median line and parallel with it, was made through the skin, and the subcutaneous space was entered. The mandren from thoracic drain No 16, using rotating and rectangular movements, partly sharply partly bluntly, tunneled the channel to the dorsal part of the neck, between the ears, where the exit site was made. The catheter was then fixed to the mandren and carefully pulled back through the tunnel into the abdomen. Muscles were sharply or bluntly moved apart to access the peritoneum. Immediately after opening the peritoneal cavity, biopsies of parietal peritoneal tissue were taken from diagonal edges, and the catheter was placed at the bottom of the peritoneal cavity. The peritoneum was sutured with

ongoing suture using Vicryl 4-0, and the catheter was fixed to the peritoneum. Muscles were sutured with chromium Cutgut 3-0, the fascias with ongoing Vicryl (Dexon) 3-0 suture, and the skin with single sutures. The catheter was fixed in the tissue at both entering and exit site. At both sites a sterile gauze was placed and fixed with a bandage wrapped circularly around the animal.

#### *Infection prevention*

Daily injections of cefuroxime (Nilacef, Hemofarm, Serbia and GlaxoSmithKline, England) were applied in order to prevent infection of the wound and peritoneal cavity, according to the protocols from literature (Peng *et al.*, 2000; Mortier *et al.*, 2003). The antibiotic was given intramuscularly twice a day at a daily dose of 150 mg for three days prior to catheter placement and three days following catheter removal. During dialysate instillations, 75 mg of antibiotic was given through the catheter, once a day.

#### *Dialysate instillations*

There is a variety of recommendations for instillations frequencies among researchers on PD (Mortier *et al.*, 2005). In our study instillations of dialysate fluid (Dianeal PD4 Glucose, Baxter Vertriebs GmbH, Austria) with 3.86% glucose concentration, preheated at 37°C, were started 7 days after catheter insertion, once a day. Full instilled dose of 40 mL/kg of body mass was reached gradually, starting with 60 mL of dialysate, and increasing the amount of dialysate used by 10 mL each following day.

#### *Catheter obstruction prevention*

To prevent catheter clogging (De Vriese *et al.*, 2002), heparin natrium (Heparin, Galenika a.d, Serbia) was injected through the catheter, at a dose of 10 i.j. once a day.

#### *Sample collection*

Peritoneal tissue samples for histological analysis were collected strictly following the guidelines from literature (Williams *et al.*, 2002; Jorres and Witowski 2005), because peritoneal tissue is extremely fragile and susceptible to mechanical irritation and environmental factors. Ellipsoid tissue samples, 18 mm x 3 mm, of parietal peritoneum were taken just after opening the abdomen. Immediately after biopsy the tissue was fixed for 24 hours with 10% formaldehyde with 0.1M Sorensen's phosphate buffer pH 7.4, dehydrated in 96% ethanol, then routinely processed for embedding in paraplast and stained with hematoxylin-eosin and toluidin-blue to be analyzed by light microscope (Opton Photomikroskop III).

For transmission electron microscopy tissue samples were fixed for 24 hours in 4% glutaraldehyde with 1% tannic acid to ensure better fixation of membrane structures. Fixatives were diluted in cacodylate or Sorensen's phosphate buffer 0.1M, pH 7.4. The tissue was then rinsed three times for 10 minutes with the same buffer, and then three times for 10 minutes in cacodylate buffer. Samples were postfixated with 1% osmium tetroxide in 0.1M cacodylate buffer, pH 7.4 and left overnight in 4% uranyl acetate. They were then dehydrated in ethanol and propylene-oxide and embedded in Epon. Fine sections were

contrasted with uranyl acetate and lead-citrate and analyzed with transmission electron microscope (Philips M208S).

A double-blind histological investigation was carried out – two researchers independently studied and described the samples, not being aware of their origin (peritoneal tissue sampled before or after PD treatment).

All experimental procedures were performed in accordance with the European Council Directive (86/609/EEC), and were approved by the Animal Care Committee of the University of Belgrade.

#### RESULTS AND DISCUSSION

*In vivo* peritoneal dialysis research is hampered by the large variety of available models that make interpretation of results and comparison of studies very difficult. There is no consensus on the ideal experimental model so far (Mortier *et al.*, 2005), and research groups work on animal models that differ substantially according to species and strain of experimental animals (Trbojević *et al.*, 2006), method of peritoneal access (Schambye *et al.*, 1992; Zweers *et al.*, 1999), study duration, measurement of solute transport and ultrafiltration (Kim 2009), and sampling for histological analysis (Dobbie 1993; Di Paolo *et al.*, 1996; Gotloib and Shoshtak 2000; Obradović *et al.*, 2000; Stojimirović *et al.*, 2001; Williams *et al.*, 2002; Jorres and Witowski 2005).

In our study the anesthesia procedure was convenient for catheter implantation and removal. No adverse effects were noted, and rabbit's complete recovery was observed immediately after the surgery.

Animal's body weight (Figure 1) and temperature (Figure 2) did not change remarkably during the follow up.

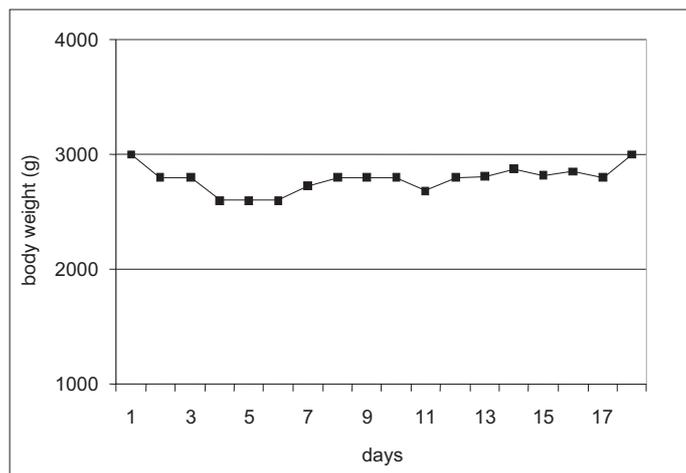


Figure 1. Rabbit's body weight during the study period

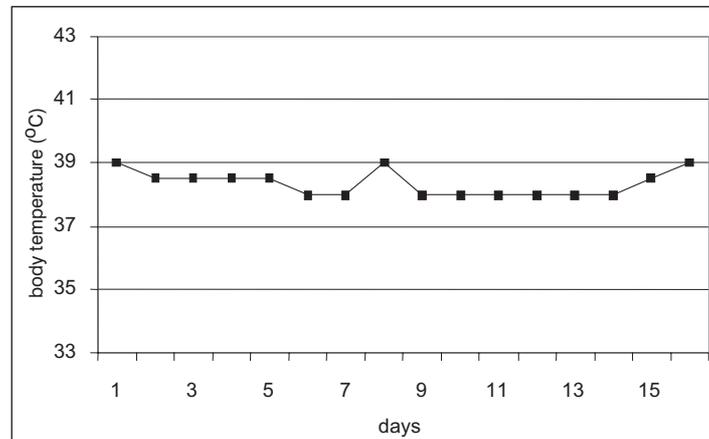


Figure 2. Rabbit's body temperature during the study period

No wound infection was observed after surgery.

The rabbit tolerated well the applied regimen of dialysate instillations, without respiratory distress. During the follow up period no signs of peritonitis were noted, indicating that the applied antibiotics offered efficient protection. No catheter obstruction occurred during the follow up period due to heparinization.

Peritoneal tissue samples were first analyzed by light microscopy (LM). The sample showed numerous blood vessels, scattered collagen fibers and occasional fibroblasts, mononuclear phagocytes and adipocytes (Figure 3).

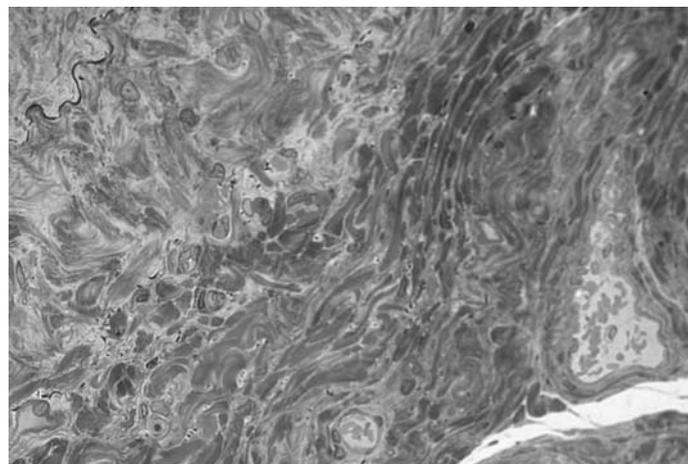


Figure 3. Tissue sample of rabbit's peritoneum (LM, × 250)

When observed with a transmission electron microscope (TEM), blood vessels walls showed a single layer of endothelial cells on a continuous single basement membrane (Figure 4). Endothelial cells were elongated, with large, predominantly euchromatic, centrally positioned nuclei. Mitochondria, ribosomes, pinocytotic vesicles, prominent rough endoplasmatic reticulum, well-developed Golgi apparatus and nuclei were observed in the endothelial cytoplasm. Pericytes with long, foot-like processes, were also observed.

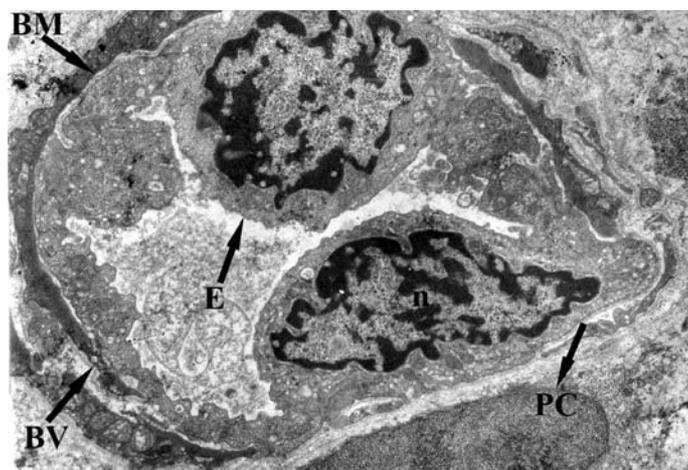


Figure 4. Blood vessel in rabbit's peritoneum (TEM,  $\times 30000$ )

BV – blood vessel; E – endothelial cell; BM – basement membrane; PC – pericyte;  
n – nucleus

Increasing use of PD in treating patients with end-stage kidney disease imposed the need for better understanding of a healthy peritoneal structure and physiology, as well as changes caused by dialysis (Stojimirović *et al.*, 2002). Studies on humans are connected with numerous technical and ethical problems and restrictions. It is technically complicated to perform biopsy of diaphragmal and visceral peritoneum, as these parts are not easily accessible during standard surgical procedures of the front abdominal wall for placement or removal of the PD catheter. Such samples can only be obtained during excessive abdominal surgeries, which are painful and traumatic for the patient and impede obtaining adequate samples of fragile peritoneal tissue. The main ethical problem is performing peritoneal biopsies on healthy persons. When acquiring tissue samples from humans, special care must be taken not to disturb the person's comfort. Furthermore, peritoneal tissue is very fragile and deteriorates rapidly when exposed to air during the surgical intervention, as well as in the period between biopsy and fixation (Trbojević *et al.*, 2006).

Though efforts are made to study dialysate effects on the peritoneum using cell cultures, there is no doubt that animal models, particularly those using rabbits, are the best option for investigating this problem.

The here presented modified non-uremic rabbit model of peritoneal dialysis is suitable for obtaining peritoneal tissue samples for light and transmission electron microscopy examination, and can be used to analyze the effects of different dialysis solutions on rabbit peritoneal membrane.

ACKNOWLEDGEMENT:

The study was supported by research grant from Ministry of Science and Environmental Protection Republic of Serbia N<sup>o</sup> 145070.

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## PRVI HISTOLOŠKI NALAZI NA MODELU PERITONEALNE DIJALIZE KOD KUNIĆA

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### SADRŽAJ

Istraživanje promena na peritoneumu koje nastaju pod uticajem rastvora za dijalizu tokom peritonealne dijalize, predstavlja još uvek izazov u humanoj medicini. Ova studija je imala za cilj da obezbedi uzorke peritoneumskog tkiva kunića pogodne za histološke analize, korišćenjem modifikovanog infuzionog modela peritonealne dijalize na neuremičnim kunićima. Dvolumenski centralni venski kateter, hirurški plasiran u peritonealnu duplju odraslog Činčila kunića, korišćen je za svakodnevna instilacija peritonealnih rastvora. Uzorci peritoneuma tkiva dobijeni su tokom plasiranja i uklanjanja katetera i analizirani svetlosnom i transmissionom elektronskom mikroskopijom. Rezultati histoloških ispitivanja su dokazali da je korišćen modifikovani infuzioni model peritonealne dijalize na neuremičnom kuniću adekvatan za dobijanje uzoraka tkiva peritoneuma za ispitivanje svetlosnom i transmissionom elektronskom mikroskopijom i da može da se koristi za istraživanje efekata različitih dijaliznih rastvora na peritonealnu membranu kunića.