Animal Models in Peritoneal Dialysis

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Summary

Over the last decades peritoneal dialysis (PD) has become a successful and widely used treatment for end-stage renal disease patients worldwide. Together with the increasing number of uremic patients successfully treated with PD has grown an interest in physiological, pathophysiological and clinical aspects of this therapeutic method. This article provides an overview of the current status on animal models used in studying the histology and physiology of the peritoneum, as well as the process of peritoneal dialysis itself. We discuss species of experimental animals, methods of peritoneal access, sampling for histology, different techniques and methodologies, and complications of experimental models of PD.

Introduction

Peritoneal dialysis (PD) is a treatment method for patients with end-stage renal insufficiency. Its efficacy and possibility for at-home performance make it the treatment of choice for many patients (*Blake, 2001; Trbojevic et al. 2001*). It is especially recommended for elderly persons, patients with insulin-dependent diabetes or heart disease (*Stojimirovic and Nesic, 1998*). The main goal of this treatment is to achieve complete social and physical rehabilitation of the patient.

The main elements of the PD system are the peritoneal vascular network, peritoneal membrane and dialysis solution in the peritoneal cavity. Dialysis solution is instilled continuously or intermittently into the peritoneal cavity through a surgically implanted catheter in the front abdominal wall. Water and solute exchange between this solution and the patient's blood is achieved by osmosis and diffusion. These processes occur through the peritoneal barrier consisting of a stagnant fluid layer within the peritoneal capillaries, the capillary endothelium and basement membrane, the interstitium, the mesothelium and the stagnant fluid layer within the peritoneal cavity. In this way PD substitutes at least two of the impaired renal functions in uremic patients: evacuation of degradation products accumulated in the blood and removal of excess water (*Stojimirovic and Nesic, 1998*).

Mesothelium is damaged by uremic changes in the internal environment, while PD additionally injures the peritoneum (*Obradovic et al., 2000; Stojimirovic et al., 2000*). Continuous exposure to hyperosmolar dialysis solution with low pH and high glucose concentration causes various pathological changes in the peritoneal membrane structure. Frequent peritonitis episodes further damage this fragile tissue (*Stojimirovic et al., 2001a, 2001b*).

Limitations in researches of human peritoneum and advantages of animal models

Increasing employment of PD in treating patients with end-stage renal failure imposed the need for better understanding of the structure and physiology of healthy human peritoneum, as well as changes caused by PD the process (*Stojimirovic et al., 2002*). Researches on human material, however, carry a number of technical and ethical problems

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and limitations. Technically, it is complicated to perform biopsy of diaphragmal and visceral peritoneum since these regions are inaccessible during standard surgical procedures on the front abdominal wall for placement or removal of a PD catheter. Such samples can only be obtained upon excessive abdominal surgery, which are painful and traumatic for the patient and impede obtaining a valid specimen of fragile peritoneal tissue. The main ethical problem is obtaining peritoneal biopsy samples from healthy persons. Furthermore, when collecting material from humans the person's comfort is an important concern, as is the fragility of peritoneal tissue, which rapidly deteriorates when exposed to air during the intervention and in the time between biopsy and fixation (*Trbojevic, 2004*).

The ideal methodology for studying changes in the peritoneum in connection to PD treatment would be sampling of healthy peritoneum, and then prospective biopsies on the same person in different stages of renal disease, and later, during PD treatment. Such approach, although ideal from methodological perspective, is impossible in practice for ethical and technical reasons. Therefore, there are no data in available literature on serial, prospective studies on humans (*Di Paolo and Sacchi, 2000*). Furthermore, in our country it is still virtually impossible to follow one person through the stages of renal disease, as there is still no national database on patients.

Due to the mentioned ethical and technical restrictions, the process of PD and changes in tissue structure have, so far, mostly been studied on animal models (*Trpinac et al., 1998*). These models enable *in vivo* and *ex vivo* research of healthy peritoneal membrane, changes appearing during dialysis, biocompatibility of dialysis solutions and production of new, and improved dialysis solutions.

Animals

Peritoneal structure is similar in all mammals thus justifying generalization of results obtained from animal models on humans. Researchers mostly use small mammals – rats and rabbits.

Rats are economical, easy to obtain and keep (*Lameire et al., 1998*). Their main flaw is short life expectancy when on PD and dialysis duration is crucial for development of typical histopathological alterations of peritoneal tissue. When interpreting results, the ratio between human and rat life expectancy must therefore be taken into consideration. As the average total life span of a rat is 2.5 years, 16 to 20 weeks of dialysis might be similar to 5 to 10 years duration of PD in humans (*Topley, 2005*). Due to a rat's small size, peritoneal catheter implantation is difficult so the dialysis solution is often introduced into the peritoneal cavity by blind abdominal punction. This approach carries the risk of perforation of internal organs, introducing infection and the quantity of PD solution is small and the dialysate is difficult to recover, which results in small quantities of the effluent being available for analysis. Another important issue is the ratio of peritoneal surface area to exchange volume of dialysis fluid, which is significantly higher in rats than in humans (*Garosi and di Paolo, 2001*).

Experimental models of PD in uremic rabbits had already been established in the early 1980s where uremia had been induced by subtotal nephrectomy (*Lameire et al., 1998*). Recently, several non-uremic rabbit models of PD have been described (*Zweers et al., 1999; Zunic-Bozinovski et al., 2007*). Rabbits survive longer on PD and mortality from peritonitis is lower (*Garosi and Di Paolo, 2001*). The catheter is easily inserted, with exit site on the dorsal part of the neck. Exchanges of dialysis solution are performed as in humans. The ratio of peritoneal surface to exchange volume in rabbits and humans is similar. On the other hand, rabbits are more delicate then rats and more difficult to breed (*Garosi and Di Paolo, 2001*).

More recent studies use genetically modified mice. In these cases, however, animal size significantly complicates manipulation (*Ni et al., 2003*).

Practical aspects of animal models in peritoneal dialysis

Development of an adequate animal model of PD for studying peritoneal structure and physiology, as

well as the dialysis process itself, still remains a challenge. The main goal is to design the model, which would adequately imitate the process of PD in humans and provide information for studying the structure and physiology of peritoneal membrane, as well as the process of peritoneal dialysis *per se*, pathophysiology of peritoneal transport, structural changes and local peritoneal defense mechanisms.
A variety of chronic PD models have been developed by different research groups making interpretation of the results and comparison of studies very difficult (*Lameire et al., 1998; Topley, 2005*). There is therefore an evident need for consensus in methodological approach.

Peritoneal access

So far, three techniques for instillation of dialysis fluid into an animal's peritoneal cavity have been used. Some researchers introduce the solution into rat's peritoneal cavity by blind punction of the front abdominal wall with a 22G needle (*Peng et al., 2000*). Repeated punctions may cause intraperitoneal bleeding, infection or trauma to peritoneal tissue, all of which can interfere with experimental results. Use of anesthetic can influence peritoneal permeability and kinetics of peritoneal transport by its effect on lymph drainage (*Tran et al., 1993*). Another method, the so-called "opened" permanent system, uses instillation and removal of dialysis fluid through a catheter placed in the tunnel from the neck to the peritoneal cavity (*Pawlaczyk et al., 2001*). This method does not require anesthesia, but the risk of infection is higher, as well as the risk of catheter obstruction due to omental wrapping, adhesions and fibrosis (*Wieczorowska-Tobis et al., 2001*). The third, so-called "closed" system, method has been introduced recently. A permanent catheter is tunneled from the peritoneal cavity to the neck and connected to a subcutaneous port. Dialysate draining via the catheter is not possible in this model, so it is left in the peritoneal cavity to be absorbed. This approach reduces the risk of infection, but catheter malfunction due to obstruction still remains a problem (*Zweers et al., 2001*).
Peritoneal access in rabbits is somewhat simpler and is usually achieved via a permanently implanted catheter. Most researches use double-lumen central venous catheter (*Zweers et al., 1999*), but our group suggested an easily adapted infusion system as an excellent alternative (*Zunic-Bozinovski et al., 2007*).

Instillation volume, instillation frequency and exposure period

Humans have a peritoneal surface area of approximately 17 000 cm^2, and Wistar rats with body weight 350 g have a peritoneal surface area of about 600cm^2. Therefore, an instillation volume of 70 ml in the rat would be proportional to the quantity used clinically in humans (Rubin *et al.* 1988, Di Paolo *et al*. 1995). However, due to rat respiratory compliance and in order to avoid leakage, only 30 – 40 ml can be instilled. In rat models of PD most often used, the instillation volume is 10 ml (*Mortier et al. 2005, Zaerie et al., 2005*). Rabbits are usually instilled with 40 ml/kg dialysis solution, which is more proportional to the volume used in humans (*Struijk, 2001*). However, we recommend that the full dose is reached gradually to avoid respiratory problems caused by introduction of a large amount of fluid (*Zunic-Bozinovski et al., 2007*).
Frequency of instillation varies among different research groups, ranging from once to three times daily (*Fracasso et al., 1999; Peng et al., 2000; Margetts et al., 2001; Mortier et al., 2004*). Repeated exposures more closely resemble to the multiple-exchange program performed in PD patients.
So far, there is no consensus on the optimal exposure period needed for development of specific alterations of peritoneal membrane. After catheter implantation, a nonspecific inflammatory reaction develops which stabilizes in about 3 weeks (*Wieczorowska-Tobis et al., 1997*). Some authors suggest that treatment duration of at least 12 weeks is necessary for development of significant differences in the effects of different dialysis solutions on peritoneal membrane (*Mortier et al., 2004*).

Complications

The most important technical problems in all types of animal models remain catheter obstruction and high incidence of peritonitis.

To avoid mechanical catheter obstruction, some investigators perform omentectomy before implantation or add heparin to the test solutions to reduce formation of peritoneal adhesions (*Pawlaczyk et al., 2001; Wieczorowska-Tobis et al., 2001*). However, having in mind the role of omentum as defense organ, as well as the fact that heparin exerts effects beyond anticoagulant, such as modulation of the activity of inflammatory cells, synthesis of the extracellular matrix, proliferation of cells and neoangiogenesis, neither of these approaches is completely satisfying. Thus, the use of heparin-coated catheters seems to be the preferable method of peritoneal access, as it largely prevents obstruction with no influence on peritoneal membrane (*De Vriese et al., 2000; De Vriese et al., 2002*).

Peritonitis is diagnosed by dialysate culture or white blood cell counts. The critical white blood cells count for diagnosis of peritonitis varies from study to study, so the combination of a positive dialysate culture and a dialysate white blood cell count higher then 1 000 cells/mm^3 is arbitrarily defined as peritonitis (*Mortier et al., 2003*). In studies using an "open" catheter system, peritonitis incidence varies from 0.23 – 0.5 episodes/animal/month, while for "closed" systems there are no consistent data. Prophylactic administration of antibiotics during the study period adequately prevents infection and associated alterations of peritoneal structure and function (*Mortier et al., 2003*).

Tissue sampling

Irrespective of the type of experimental model and the choice of the animal, the most important problem in studying the structure of peritoneal membrane is obtaining a valid tissue sample. Most research groups use mainly the visceral animal peritoneum. In studies on humans, however, mostly parietal peritoneum is sampled as it is more easily accessible. Analysis of paired biopsies of parietal and visceral peritoneum in humans suggests that alterations in the visceral membrane are less pronounced then in the parietal membrane. The reverse was observed in some animal studies, which justifies such an approach in tissue sampling (*De Vriese et al., 2002; Martin-Martinez et al., 2004; Williams et al., 2003*). There is presently no consensus on where exactly the representative peritoneal samples should ideally be harvested. This may be the cause for variability of the results as certain structures have variable distribution in different sections of the peritoneum (*Mortier et al., 2005*).

Another important issue is the extreme fragility of peritoneal tissue, which dries quickly when exposed to air, and reacts with ultrastructural changes even to a light touch of a surgical glove. The sample must therefore be obtained and fixed immediately following the opening of the abdominal cavity (*Di Paolo et al., 1995*). Prior to the biopsy, the animal is anesthetized. Intraperitoneal administration of anesthetic may influence peritoneal structure and function, subcutaneously applied anesthesia acts slowly, and the intramuscular route is prohibited in some countries (*Wieczorowska-Tobis et al., 1997; Zweers et al., 2001*).

Methodologies for studying alterations of peritoneal tissue

Semiquantitative method

This method is used in testing biocompatibility of dialysis solutions. It is based on subjective evaluation of experienced investigators if certain alteration is present in the tissue or not. Although widely used, this approach has obvious limitations. Besides subjectivity in assessment, certain changes can only be defined as present or absent, with no possibility for quantification (*Di Paolo et al., 1995*).

Histomorphometry

Semiautomatic systems for histomorphometry are image analyzers which provide accurate measures of distance and area on digital images of tissue obtained with a digital camera connected to a light

microscope. They provide precise quantitative morphometric analysis of histological elements of the peritoneum and alterations, which appear due to PD and are especially useful for comparing the biocompatibility of PD solutions. The method can quantify the percentage of cubic mesothelial cells, submesothelial oedema, lumen perimeter and dimensions of different layers of peritoneal blood vessel wall (*Garosi et al., 2001*).

Intravital microscopy

This technique is a sophisticated research tool to evaluate the function and structure of a living tissue. It allows the *in vivo* measurement of peritoneal blood flow rate, microvascular permeability to macromolecules, leucocyte-endothelial interactions, microvascular density and lymph vessels kinetics. Instead of biopsy, a small midline abdominal incision is made and a short segment of the visceral peritoneum is exteriorized, spread over a Plexiglas slide and superfused continuously with an isotonic, isocolloidal solution. The tissue is then observed with a light microscope using water immersion. The method uses commercial hardware and software (*De Vriese et al., 2001*). Contrary to previously described methods, this one can not be performed on humans.

Conclusion

The experimental animal models of PD show significant development in the recent years. There are, however, still many questions awaiting answers, the most important one being - to which extent can the observations on animals be translated to the human situation?

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