ANIMAL MODELS OF PERITONEAL DIALYSIS

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Abstract

Peritoneal dialysis is an extensively used mode of renal replacement therapy. A lot of research in relation to peritoneal membrane transport characteristics, inflammation and peritonitis and the use of biocompatible dialysis solution is done in animal models. Small animals such as rats, rabbits, and genetically modified mice are mostly been used as experimental models, though larger animals such as dogs, sheep, or even kangaroos are being used. The size of parietal peritoneum and peritoneal surface area in rabbits are comparable to humans and hence are considered as ideal model and they can survive longer on peritoneal dialysis. The biocompatibility testing of dialysis fluids and effects of uremia are extensively studied in animal models. A few pitfalls with animal models have also been identified.

Peritoneal dialysis (PD) is a well-established and extensively used method of kidney replacement therapy for patients with end stage renal disease. It is currently estimated that there about 200,000 patients on peritoneal dialysis worldwide at present and it constitutes 11% of the total number of patients receiving some modality of dialysis [1].

A lot of research and in depth understanding of the physiology of the peritoneal membrane, the transport of solute and water across it and the mechanisms influencing inflammation, peritoneal injury and encapsulating peritonitis is necessary. The newer PD solutions, and pharmaceutical agents needs to be tested before being implemented in the daily clinical practice. Trials on humans is complicated with technical problems and ethical concerns. The scientific community in order to overcome this problem has exploited the observed similarities among humans and animal models. The similarity in transport properties of solute and water across the peritoneal membrane in humans and animal models was an important foundation for the conduction of series of

experiments in a variety of animal models. It is Putman T in 1923 who made the first attempt for PD in a canine model [2].

Small animals such as rats, rabbits, and genetically modified mice are mostly been used as experimental models, though larger animals such as dogs, sheep, or even kangaroos have been used. The use of each animal model comes with different advantages and disadvantages.

Small animals -

Rats –

Rats are easy to breed and affordable, but they have a short life expectancy. They are small sized that increases the risk of complications during the insertion of a peritoneal catheter and only small quantities of dialysate can be introduced in their peritoneal cavity. Rats have a large peritoneal membrane surface area compared to humans [3].

Rabbits –

Rabbits have a longer life expectancy, can survive longer on PD and the insertion of the peritoneal catheter is easier but are very delicate animals and difficult to breed [4]. The size of parietal peritoneum and peritoneal surface area in rabbits are comparable to humans and hence are an ideal model however, their breeding is difficult and expensive and a large time frame is needed for results to be obtained [5].

Genetically modified mice -

The genetically modified, knockout and transgenic mice models have been used to gain insight into the different molecular pathways that are significant for peritoneal dialysis [6]. The role of single proteins such as aquaporin-1 in the transport of water across the peritoneal membrane [7] or NOS (Nitric oxide synthase) isoforms in peritonitis [8], as well as, IL-6 in inflammation [9] and TGF-b in encapsulating peritonitis [10] were explored using genetically modified mice. The extremely small size of these animal makes manipulations difficult, but the recent development of new molecular techniques provide significant tools to overcome this disadvantage [11]. The advantages of mice

include the easy and affordable breeding, fast generation and maturation and the Ability to explore the role of single proteins.

Large animals –

The large animals used as models include dogs, sheep, kangaroos etc. They have longer life expectancy and the peritoneal catheter insertion is easy. However their breeding is difficult and expensive and results take a longer time.

Acute models -

The acute models describe experiments that require a shorter duration and can study the impact of a single dwell on the peritoneal membrane. They can provide information regarding the transport characteristics of the peritoneal membrane and its function. Its permeability to water and solute, the interaction with different dialysis solutions, therapeutic agents or even inflammation mediators can be extensively studied this way.

In the acute model the animal is anesthetized, a temporary catheter is inserted in the peritoneal cavity and during a 4-hour dwell samplings of the peritoneal fluid and blood are obtained [12] .

Chronic models -

The chronic animal models can evaluate the long term impact of peritoneal dialysis. They focus on changes of the structure and the function of the peritoneal membrane example, the effect of glucose used as an osmotic agent in dialysis solutions or the effect of the newer biocompatible solutions with lower GDPs' (Glucose degradation products) concentration, neutral pH or replacement of glucose with icodextrin on the morphology of the peritoneal membrane can be analyzed. Fibrosis, encapsulating peritoneal sclerosis, repeated episodes of peritonitis, and morphological alterations can be in depth studied using the chronic models. The targeted therapeutic interventions are studied using the chronic models.

In the chronic model, a permanent indwelling peritoneal catheter from the neck to the peritoneal cavity of the animal is inserted subcutaneously. This is called "open system" and can be used for the introduction of dialysate into the peritoneal cavity and the drain

of the dwell directly or indirectly as it can be used as the tunnel through which another sterile catheter is inserted during every exchange. In this method anesthesia is not required but the rate of peritonitis episodes remains high and the malfunction of the catheter as a result of omental wrapping, adhesions and fibrosis is frequent. Finally, there is a "closed system" in which a permanent indwelling peritoneal catheter is inserted subcutaneously from the neck to the peritoneal cavity of the animal. The catheter is then attached to a subcutaneous reservoir in the neck of the animal and the dialysate remains in the peritoneal cavity until it is fully absorbed. This method results in lower rates of peritonitis.

Biocompatibility testing of dialysis fluid in animal model -

The in vivo biocompatibility tests allow us to evaluate new dialysis solution in the complex system of the living organism. Exposure of the peritoneum to the tested fluid usually lasts for days and weeks, and so provide sufficient time for the development of pathological changes such as fibrosis and neovascularization of the peritoneal interstitium.

There is no standard experimental approach to the testing of biocompatibility of dialysis fluids in animals. Gotloib et al [13] used "imprints" of the mesothelial cells removed from the surface of the peritoneum; the "imprints" provide information about changes in the mesothelial cells induced by their repeated exposure to dialysis fluids. They evaluated parameters such as mesothelial cell viability, size of the cells, number and sizes of nuclei, and activity of the cellular enzymatic systems.

Slater et al [14] demonstrated hyperplasia of the mesothelial cells in rat peritoneum chronically exposed in vivo to dialysis fluids and found that transperitoneal ultrafiltration decreases with enhanced absorption of glucose from the dialysate.

Di Paolo et al [15] suggests that animal studies of biocompatibility should extend for at least three months . The parameters to be measured during such experiments, include morphology of the peritoneum, constituents of the dialysate that reflect peritoneal permeability, and biosynthetic function (phospholipids), and products of peritoneal leukocytes (interleukins). The phospholipid levels in the dialysate might provide a sensitive index of peritoneal function according to them.

Effect of Uremia in animal models -

Gotloib et al [16] described a model where rabbits were made uremic by unilateral nephrectomy and 5/6 nephrectomy on the opposite side and were initiated on peritoneal dialysis. In these animals, CAPD gave adequate control of the uremia, but body weight and total serum protein concentration decreased. Uremic animals were maintained on peritoneal dialysis for up to 14 days with good control of urea nitrogen. However, this study does not provide any evidence of whether uremia influenced the permeability of the peritoneum or the function of peritoneal host defenses. Miller et al [17] maintained uremic rats on dialysis for 21 days, during which time they observed a decline in plasma albumin and total protein concentration in dialysed animals.

Animal models for encapsulating peritoneal sclerosis -

Several different reagents have been used to alter the peritoneal tissue mimicking the tissue histology of peritoneal fibrosis. The reagents that have been used include chlorhexidine gluconate (CG), acidic (pH 3.8) glucose solution, Glucose degradation product like methylglyoxal (MGO). These agents produce inflammation and angiogenesis in peritoneum. The subsequent myofibroblast proliferation by several cytokines and growth factors, and the accumulation of extracellular matrix in peritoneum are key to peritoneal fibrosis.

The CG model -

Among the several experimental rodent models of EPS, the most commonly used due to its ease of use and adaptability, is the CG model, prepared with 0.1% CG (chlorhexidine gluconate) and 15% ethanol. CG is a chemical irritant and its repeated injections cause mesothelial cell degeneration and inflammatory responses that lead to excessive fibrosis. Inflammation cells, such as macrophages, α-SMA-positive myofibroblasts, and newborn blood vessels were observed in the submesothelial compact zone; these findings resemble those of peritoneal dialysis patients. Because the mechanisms to create peritoneal fibrosis in the CG animal model differ from those that operate in human EPS patients, more refined animal models have been needed

The MGO model -

GDPs (glucose degradation product) and AGEs (advanced glycosylation end products) play an important role in peritoneal fibrosis, and GDPs have been used to develop animal models. MGO (methylglyoxal) is an extremely toxic GDP in PD fluid and a potent promoter of AGE formation. AGEs induce inflammation and angiogenesis, as described above. Compared to the CG model, virtually the same histological features, such as thickening of the submesothelial compact zone, the presence of inflammatory cells, and neovessels, could be observed in the MGO-induced peritoneal fibrosis model . In addition, the fibrin deposition in the MGO model was greater than that in the CG model. In the MGO-induced model, the AGEs confirmed in peritoneal tissue with immunohistochemistry were similar to those of EPS patients. Hirahara et al. [18] reported that mesenchymal-like mesothelial cells, which are typically found in EPS, were observed in the MGO model, but not in the CG model. Therefore, the MGO model more closely resembles the mechanism of actual EPS than does the CG model.

Pitfalls in animal models -

The area of the diaphragm, which seems to play an important role in lymphatic drainage from the peritoneal cavity which is responsible for clearance of macromolecules, is relatively larger in humans than in experimental animals [19 20]. On the other hand, the parietal peritoneum that is directly related to the solute transport is larger in rats than in humans, while in rabbits it is similar to humans [19]. The high intraperitoneal levels of amylase in rats can locally degrade icodextrin, an osmotic agent used in several dialysis fluids, rapidly [21]. The changes in the permeability and the surface area of the peritoneal membrane are seen in rats as they grow up, resulting in changes in the kinetics of the peritoneal membrane [22].

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