Video Article

Surgical Techniques for Catheter Placement and 5/6 Nephrectomy in Murine Models of Peritoneal Dialysis

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Abstract

Peritoneal dialysis (PD) is a renal replacement therapy consistent on the administration and posterior recovery of a hyperosmotic fluid in the peritoneal cavity to drain water and toxic metabolites that functionally-insufficient kidneys are not able to eliminate. Unfortunately, this procedure deteriorates the peritoneum. Tissue damage triggers the onset of inflammation to heal the injury. If the injury persists and inflammation becomes chronic, it may lead to fibrosis, which is a common occurrence in many diseases. In PD, chronic inflammation and fibrosis, along with other specific processes related to these ones, lead to ultrafiltration capacity deterioration, which means the failure and subsequent cessation of the technique. Working with human samples provides information about this deterioration but presents technical and ethical limitations to obtain biopsies. Animal models are essential to study this deterioration since they overcome these shortcomings.

A chronic mouse infusion model was developed in 2008, which benefits from the wide range of genetically modified mice, opening up the possibility of studying the mechanisms involved. This model employs a customized device designed for mice, consisting of a catheter attached to an access port that is placed subcutaneously at the back of the animal. This procedure avoids continuous puncture of the peritoneum during long-term experiments, reducing infections and inflammation due to injections. Thanks to this model, peritoneal damage induced by chronic PD fluid exposure has been characterized and modulated. This technique allows the infusion of large volumes of fluids and could be used for the study of other diseases in which inoculation of drugs or other substances over extended periods of time is necessary.

This article shows the method for the surgical placement of the catheter in mice. Moreover, it explains the procedure for a 5/6 nephrectomy to mimic the state of renal insufficiency present in PD patients.

Video Link

The video component of this article can be found at https://www.jove.com/video/56746/

Introduction

Kidney Function and Renal Disease

Kidneys are essential organs involved in homeostasis, blood filtration and hormone production. There are various conditions that lead to kidney failure and to the subsequent onset of uremia, which has been defined as the group of systemic symptoms due to the accumulation of waste products in the blood retained due to kidney function disorders[1]. Moreover, since homeostatic capability is also affected when there is a renal failure, hypertension due to volume overload may occur, which is also dangerous as it can lead to heart failure[1]. When the functional capability of kidneys is less than 10%-15%, the patient must undergo one of the following therapeutic options: hemodialysis, peritoneal dialysis (PD) or renal transplantation.

PD is an interesting option that allows patients to continue treatment from the comfort of their home or practically anywhere, thus avoiding the need for frequent hospital visits and stays. The PD technique eliminates small toxic molecules and excess water generated by the body[2] through the instillation of an osmotic fluid (peritoneal dialysis fluid, PDF) into the peritoneal cavity. This instillation generates the osmotic gradient necessary for the exchange of solutes and water between the peritoneal capillary and PDF, a process known as ultrafiltration (UF).

Peritoneal Injury Induced by Peritoneal Dialysis

The peritoneal cavity is covered by a membrane (PM) composed of a monolayer of mesothelial cells resting on a matrix, which also houses few blood vessels, fibroblasts, macrophages and other cell populations. Unfortunately, the peritoneal membrane always suffers some alterations during PD treatment, such as apoptosis and loss of mesothelial cells, mesenchymal transition of mesothelial (MMT) and endothelial (end-MT) cells, recruitment of inflammatory cells and fibrocytes, vascular alterations, angiogenesis, lymphangiogenesis and/or fibrosis[3,4,5,6,7,8,9]. These

alterations are responsible for the development of an UF capacity failure[10], which precludes the continuation of the therapy, requiring that the patient must receive an alternative treatment to survive (hemodialysis or renal transplantation). Therefore, for these patients, it is essential to delay or control the development of these peritoneal alterations.

It has been speculated that uremia alone may cause inflammation[11], but the most important local factor is PDF bioincompatibility. Most PDFs use glucose as the osmotic agent, which causes inflammation. Due to PDF storage times and sterilization, glucose suffers a process of degradation, and new products from this reaction appear, generating more inflammation, MMT and apoptosis[12,13]. Moreover, there is also the possibility of mechanical damage due to the instillation method. All these factors, acting continuously, may generate a persistent and recurrent inflammatory state, leading to chronic inflammation, which drives to membrane deterioration and, conclusively, UF failure. How this damage could be reduced or avoided is still a matter of study.

Analyzing the Development of Lesions: From Human Samples to Animal Models

Working with human biopsies is a limiting factor due to the difficulty in obtaining tissue samples. These samples can only be obtained from surgeries performed due to catheter malfunction or transplantation, usually after years of PD treatment. This approach is useful for the analysis of pathological changes suffered by a peritoneal membrane exposed to PDF, but is not sufficient to study the development of the process. Another possibility is to analyze cells drained from dialysis effluent, but this still fails to provide a complete scenario. Merging both techniques is only possible with animal models. The peritoneal structure is similar among mammals, and therefore there are models with different animal species. There are a few studies based on sheep (Rodela *et al.*[14] and Barrell *et al.*[15]) and rabbit[16,17] models; however, smaller animals are preferable as they are easier to house and maintain, and are also more economical. The use of rats[18,19,20,21,22,23,24] offers a shorter treatment time needed to observe morpho-functional alterations. It has represented a very useful model to explore different issues such as the effect of anti-fibrotic drugs as for example BMP-7 (bone morphogenic protein-7)[25] and RAS (renin-angiotensin system) targeting[26,27,28].

However, the murine model has emerged as an ideal model with many benefits over others. The most interesting advantage is the possibility of using genetically modified mice to study the molecular and cellular basis of peritoneal damage. In fact, mice are often employed for the analysis of numerous diseases, as there are many different strains with various well-known genetic backgrounds. Other advantages include the reduced space required for housing, reduced cost of experiments (due to the animals' smaller size), ease of handling, the availability of reagents and the increasing amount of available information on the different strains of mice since they have been most commonly used animals in research.

A mice-based model employing an implanted device has been the most recently established model for PD[29,30], and has been shown to mimic peritoneal deterioration suffered by PD patients due to exposure to PDFs. This model has collaborated to understand the pathological processes implicated[31,32,33]. Moreover, it has been used to validate various potential treatments for ameliorating this deterioration using immune modulators and anti-inflammatory drugs and other anti-fibrotic and anti-angiogenic agents, such as COX-2 (cyclooxygenase-2) inhibitors[34], PPAR-γ (peroxisome proliferator-activated receptor-γ) agonists[35], Tamoxifen[36], Paricalcitol (a vitamin D receptor activator that modulates the immune reaction)[37], Rapamycin[38] and Nebivolol[39].

Developing the Mouse Model with an Implanted Catheter

The goal of this model is to resemble, as much as possible, the technique used in human PD patients, allowing to perform extended treatments of PD in small animals. So far, three techniques for instillation of dialysis fluid into the peritoneum have been tested in mice. The first one, blind puncture of the front abdominal wall, is controversial due to the multiple risks that it may incur, such as peritoneal damage, bleeding and, as is blindly performed, visceral puncture. The second technique is the so-called "open permanent system", in which the device for injecting the fluid is placed outside the body. This procedure is most similar to that performed in humans. However, it does not allow the development of long-term experiments, as it may increase the chances of infection, and generally requires the use of anesthesia to instill PDF, which may interfere with the results. The third technique is the "closed system". With this approach, the entire device used for fluid instillation is located inside the animal's body. Fluid is injected with a needle through an access port, which is placed subcutaneously. This procedure reduces the risk of peritoneal infection and bleeding as well as the need for anesthesia.

To study the effect of uremia in PD, a recent murine model has also been stablished[40] based on the PDF infusion model with catheter. This model brings in a novel technique to perform a nephrectomy in mice, thus reducing renal function. In the present article, a modification of the protocol employed by Ferrantelli *et al.* in 2015[40] has been developed. This new protocol allows catheter implantation while nephrectomy, reduces the length of the wound inflicted during surgery and facilitates access to the kidneys.

Protocol

All methods described here have been approved by the Institutional Animal Care and Use Committee of the Molecular Biology Center Severo Ochoa (Madrid, Spain).

Note: C57BL/6J female mice aged 12 to 14 weeks and weighing approximately 20 g at the start of the study were used. All the animals were housed under standard conditions and were given food and water *ad libitum*. Health conditions were checked daily. The material required, such as gloves, drape, catheter, suture and needles, should be sterile.

1. Placing the Catheter

Note: If the kidneys are not removed, they remain completely functional, so it does not consider the effect of uremia, thus permitting the study of PDF exposure in isolation. The surgery consists on introducing only the distal extreme of the catheter into the peritoneal cavity and placing the access port on the back of the animal, providing access to it. The procedure to place the catheter is as follows:

1. Place the mouse in an induction chamber and provide anesthesia using 4% isoflurane and oxygen with a flow rate of 0.4 L/min until loss of righting reflex.

1. Maintain the animal with 2% isoflurane in 100% oxygen with a flow of 0.3 L/min by means of a nosecone tube connected to the anesthesia apparatus. Confirm proper anesthetization by assessing muscular tone and response to stimulation.
2. Check the rate and depth of respiration during all the process. Use vet ointment on eyes to prevent dryness while under anesthesia. It is preferable if the procedures are done in a flow cabinet to ensure the maintenance of sterile conditions during surgeries.

2. Shave the right flank and the back of the animals in order to perform the surgery and to later inject the fluid at the access port in a clean area. Place the animal in a lateral position resting on its left flank in the surgical table, with a thermal system to ensure that its temperature will not fall.
3. Disinfect the area with 1% chlorhexidine gluconate solution. Make a small cut (0.5 cm) with blunt scissors in the skin on the right flank of the body, and separate it carefully with the help of the scissors from the adjacent muscle layer, so that the whole area of the back of the animal is well separated to later be able to introduce the port of access with ease. Please reference **Figure 1** to see the materials required to follow the procedure.
4. Make a small incision of about 1 mm in diameter through the muscle layer and insert the tip of the catheter and the first plastic ring. The peritoneal damage caused is minimal.
5. Suture the peritoneal wall tightly around the catheter, with a 5.0 or 6.0 non-absorbable suture. One plastic ring is then located inside the peritoneal cavity, and the other between the muscle and the skin. The catheter is thereby fixed to prevent fluid from leaking into the subcutaneous space.
6. Insert the access port into the subcutaneous space towards the tail of the mouse, without securing it to a fix position to the skin, as it could itch and the animals may scratch and bit their skin.
7. Close the wound of the skin with a 5.0 or 6.0 non-absorbable suture. Remove the inhalation anesthesia and allow the animal to recover consciousness. Do not leave the mouse unattended until it has regained sufficient consciousness to maintain sternal recumbency. When fully recovered, mouse can be returned to the company of other animals.
   Note: The experiments can begin after 4 to 7 days of postoperative recovery.
8. Provide analgesia by dissolving 3 mL of ibuprofen (20 mg/mL) in 250 mL of drinking water for the day of the surgery.
9. During the post-surgical period, check the health status of the animal daily, checking that there are no reddened areas on the skin, bristly hair or wounds.
10. Inject the fluid by holding the animal (without anesthetizing it) by the tail and grabbing the access port with one hand, and the needle with the other. Disinfect the area with 1% chlorhexidine gluconate solution before injection. It is interesting to use special needles (Huber needles), that are beveled in order to part instead of pierce the silicone septum of the access port (**Figure 1A**). Two injections per day during 40 days are enough to observe peritoneal alterations (**Figure 3**).
11. When finishing the experiment, euthanize the mouse by carbon dioxide asphyxiation or cervical dislocation.

2. Performing a 5/6 nephrectomy and placing the catheter

Note: To better resemble the situation in PD patients it is possible to perform a 5/6 nephrectomy, allowing only a residual renal function. In this case, serum samples should be taken to analyze urea levels by extracting 250 µL of blood via facial vein puncture, at least one day before starting the surgeries, at the middle of the treatment and when sacrificing the animals.It is preferable if the procedures are done in a flow cabinet to ensure the maintenance of sterile conditions during surgeries.

1. Anesthetize the mice by using isoflurane as in Step 1.1.
2. Provide analgesia with 0.1 mg/kg of Buprenorphine, injected subcutaneously at the neck of the animal and by dissolving 3 ml of ibuprofen (20mg/ml) in 250 ml of drinking water for the day before and the day of the surgery.
3. Shave the laterals and the back of the animals in order to perform the surgeries and to later inject the fluid at the access port in a clean area.
4. Perform an incision of approximately 0.5 cm in the skin, at the left side, close to the ribs, to have direct access to the left kidney.
5. Open a small incision in the muscle to take the left kidney out of the peritoneum, removing the capsule and the adrenal gland. To remove the capsule is necessary to better hold the kidney outside the peritoneal cavity.
6. Burn and cut the extremes of the kidney with a cauterizer (see **Figure 1** for materials needed).
7. Reintroduce the kidney into the peritoneal cavity and suture the wounds at the muscle and the skin with insoluble 5.0 or 6.0 suture.
8. The day after, completely remove the right kidney and insert the catheter using the same incision as to remove the kidney. Again, anesthetize the mouse with isoflurane and subcutaneously inject 0.1 mg/kg of Buprenorphine just before the surgical procedure. Also dissolve 3 ml of ibuprofen (20mg/ml) in 250 ml of drinking water the day before and the day of the surgery.
9. Make an incision in the skin of about 0.5 cm and, with the help of the scissors, separate the skin at the back of the animal from the muscle to open the space where the access port will be located.
10. Perform a cut in the muscle (about 0.3-0.4 cm) to take the right kidney out of the peritoneal cavity.
11. Remove the capsule and the adrenal gland to have better access to the kidney. Ligate kidney vein, artery, and ureter with non-absorbable 5.0 or 6.0 suture and remove the kidney completely.
12. Suture the wound at the peritoneal muscle, introducing the end of the catheter so that the muscle must remain between the two plastic rings, as explained before (step 1.5).
13. Introduce the access port into the subcutaneous space and suture the skin as explained in steps 1.6 and 1.7.
    Note: Mice should rest for at least 10 days from these surgeries to ensure that the wounds at the peritoneal muscle are completely healed and there will be no leakage into the subcutaneous space while injecting the fluid. Inject the fluid as in step 1.10.
14. When finishing the experiment, euthanize the animals by carbon dioxide asphyxiation or cervical dislocation.

Representative Results

Figure 1 shows all the materials required to follow the procedures described in protocol section. For this example, mice submitted or not to nephrectomy (8 animals per group) (**Figure 2**) were exposed during 40 days (two injections per day, waiting at least 2 hours between both) to a mixture of two different PDFs, commonly used in the clinical practice: Extraneal (icodextrin-based PDF) and Dianeal (glucose-based PDF). A group with saline solution was stablished as control (n = 6).

Mice parietal peritoneal tissues were obtained from the most distant area of the catheter. Samples were analyzed in order to compare the thickening of the membrane, as well as cell presence and mesothelial layer preservation (**Figure 3**). In this regard, thickness and cell presence are increased during dialysis, and aggravated in the nephrectomized group. Mesothelial cells also show an altered morphology since intercellular unions suffer during PD.

Serum samples were obtained from nephrectomized mice to analyze urea levels by extracting 250-400 µL of blood via facial vein puncture, at three different time-points: one day before the surgeries, middle of the treatment and end point. In the case of no nephrectomized mice, serum samples were only obtained at end point (**Figure 4**). Measurements were performed using an integrated chemistry system (See **Table of Materials**). The results show that 5/6 nephrectomy induced a uremic state, increasing ureic nitrogen levels during the course of the experiment comparing with the initial state. Moreover, when kidneys are fully functional, ureic nitrogen levels remain similar to the basal state, even in mice exposed to PDF (**Figure 4**).

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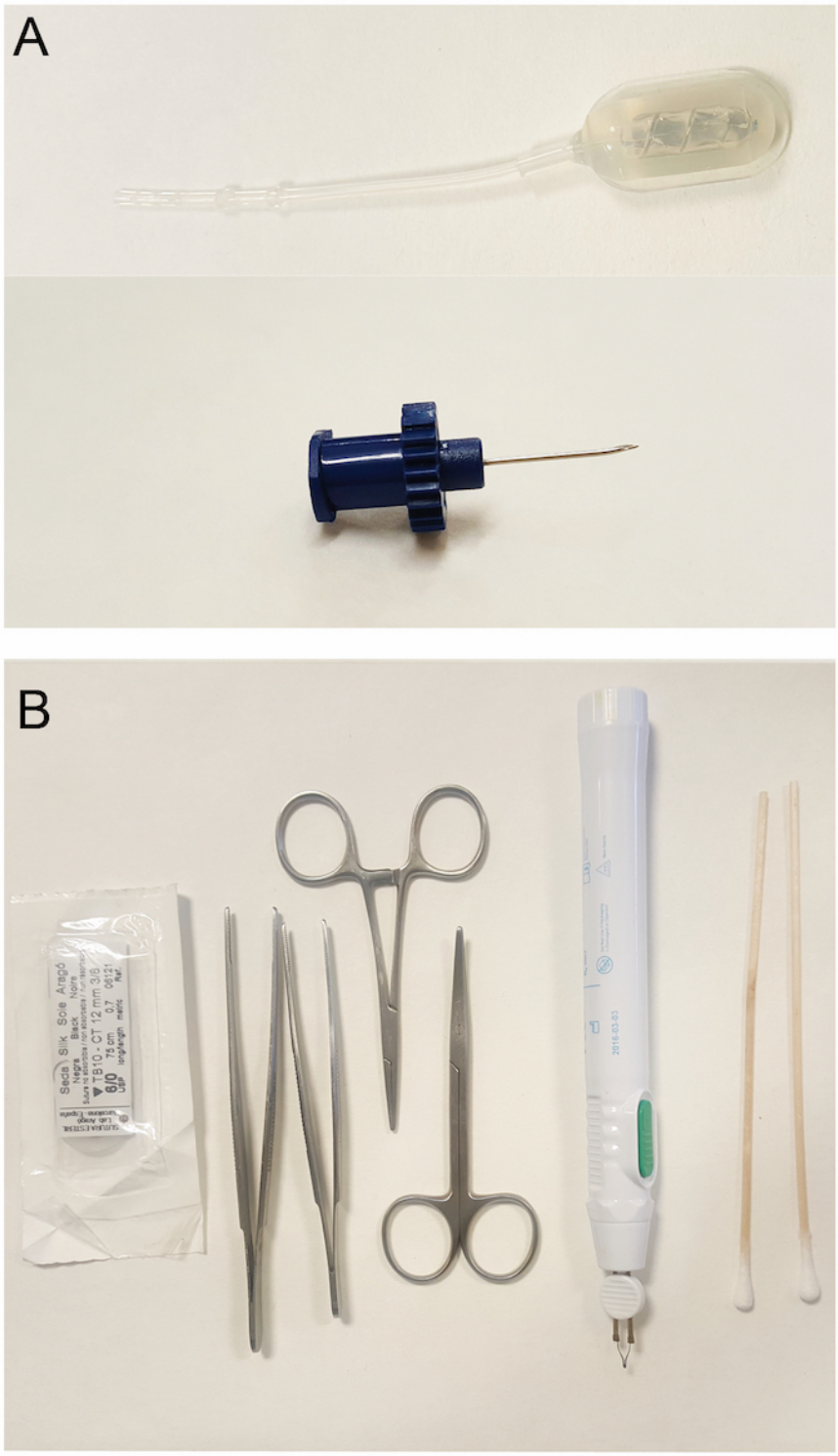

Figure 1. Material required for the surgeries of the nephrectomies and catheter implantation. (A) catheter and needle (B) 6.0 non-absorbable suture, stainless steel blunt point tweezers, locking clamp forceps (to hold the needle) and blunt scissors, cauterizer and cotton swabs. Materials should be sterilized before surgeries. Please click here to view a larger version of this figure.

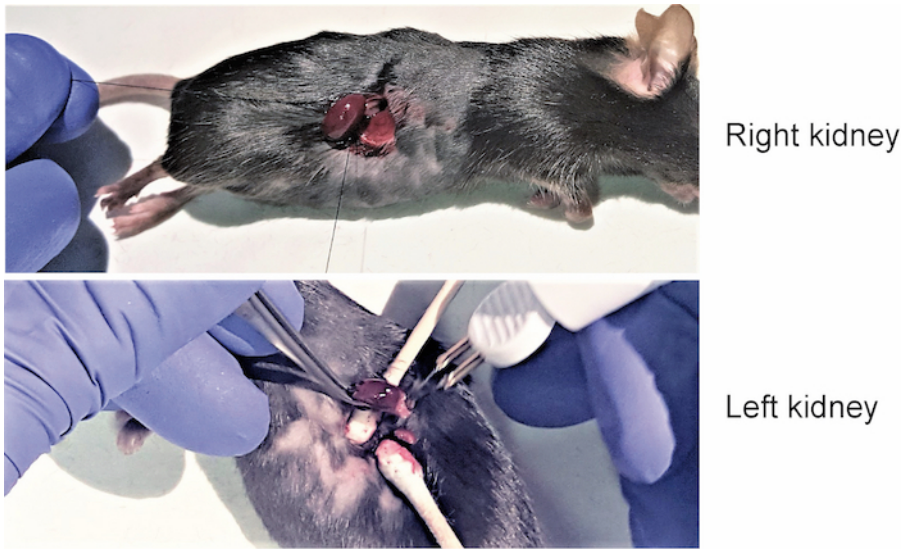

Figure 2. Pictures of the right kidney ligation and left kidney extremes removal by burning with the cauterizer. Please click here to view a larger version of this figure.

Figure 3. Masson's Trichrome staining. Representative pictures (400X) of mice peritoneal membranes exposed to PDFs + 5/6 nephrectomy, PDFs (without nephrectomy) and saline solution (without nephrectomy). Arrows show increase of cellularity and loss of mesothelial layer integrity. Black lines show the thickness of the peritoneal membrane, where the blue staining correspond to the extracellular matrix. Please click here to view a larger version of this figure.

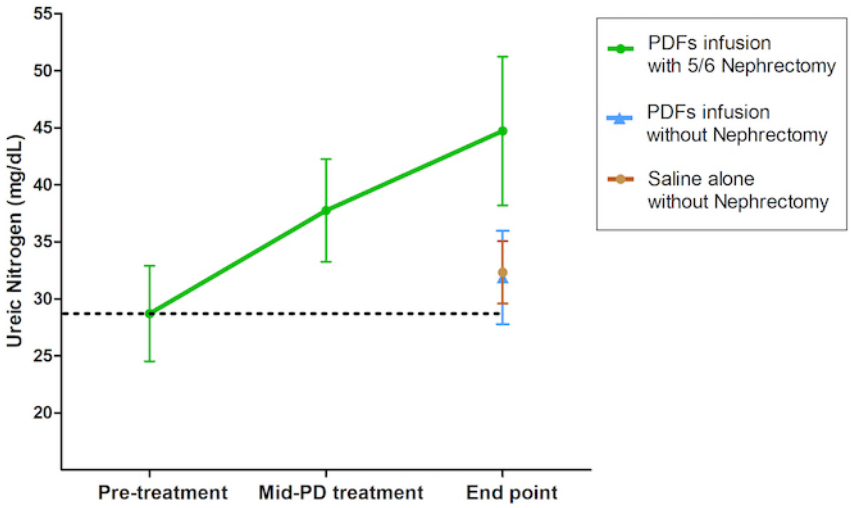

Figure 4. Serum ureic nitrogen levels (mg/dL) of mice exposed to PDF solutions, submitted or not to the nephrectomy procedure, and mice treated with saline solution alone. Data are represented as mean and standard deviation. Please click here to view a larger version of this figure.

Discussion

The first published data analyzing PD alterations by using a "close system" technique was performed in 2009[29] . This close system means that the entire device is located inside the body and fluid is injected with a needle through an access port. The most important technical problem in long-term animal models of fluid infusion through a catheter is the occurrence of obstruction. Possible options are to perform omentectomy or add heparin to the PDFs to reduce peritoneal adhesions. Nevertheless, the omentum acts as a defense organ, and heparin, apart from its anticoagulant effects, modulates processes such as inflammatory cells activity, angiogenesis, synthesis of extracellular matrix, and proliferation of cells. The design of the original device published in 2009 was later improved to overcome these problems reducing the size of the access port and adjusting the diameter of the catheter to facilitate the exit of fluid.

Animal models are essential for analyzing the evolution of numerous diseases, as well as the feasibility and potential efficacy of actions taken on pathways involved in the diseases. The peritoneal fluid infusion mouse model may be useful for studying a wide range of pathologies, as well as for developing drug therapies. This model provides an excellent tool for long-term instillation of drugs; therefore, we expect it may help improve the quality of life of patients suffering from various diseases.

There are two issues that should be taken into account for these experiments. The first one is the fact that the fluid is administered into the abdomen but not removed like in PD patients. First of all, it is important to note that these are PD-exposure models, where the aim is to study the effects of the fluid over the peritoneum, not to remove water and metabolites. Nevertheless, for the PD studies in mice there is no need for fluid removal every time, since it can be eliminated with urine. In fact, we have observed that 5/6 nephrectomized mice do not become edematous because the kidney fraction that remains is still functional and the time we leave between the daily injections is enough to urinate the administered volume. Moreover, the extraction of the fluid would involve anesthetizing the animal every day and opening the peritoneal cavity to drain it, with subsequent tissue damage. Another option could be to extract the fluid through the catheter, but it would collapse because it will suck the organs. A third option has been recently published[41], but it is not suitable for long treatments.

The second issue is that indwelling catheters may cause a foreign body reaction that could interfere with the results[42,43]. Therefore, this effect was studied in the peritoneal membrane of mice exposed only to the presence of the catheter. The results showed that there is a thickening of the peritoneum and accumulation of new cells at the insertion site. However, this reaction diminishes progressively in areas that are distant from the catheter insertion point. The peritoneal membrane on the side of the peritoneum opposite the catheter has the same appearance as the membrane of a naïve control mouse (data not shown). For this reason, it is important to analyze the left side of the peritoneal wall when looking for morphological alterations, avoiding also the *linea alba*.

The use of an indwelling catheter avoids the need for repeated punctures in the peritoneum over the duration of treatment, thus reducing the risk of infection, hemoperitoneum and the possibility of harming an organ. Furthermore, this technique more closely resembles the PDF instillation procedure in human patients. When fluid is to be injected, the animal remains completely awake. The selected area of the skin is cleaned and only the access port is held. It is therefore unnecessary to hold the animal, which may cause it undue stress, and obviates the need for anesthesia, which may interfere with the results.

The protocol for the nephrectomies firstly published by Ferrantelli *et at.* in 2015[40] has been modified to reduce the area of wound for the surgery and taking advantage of the incision necessary for the extraction of the right kidney to introduce the catheter.

Disclosures

The authors have nothing to disclose.

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