

A New Non-Uremic Rat Model of Long-term Peritoneal Dialysis

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Abstract

Together with the development of peritoneal dialysis (PD), appropriate animal models play an important role in investigation of physiological, pathophysiological and clinical aspects of PD. However there is still not an ideal experimental PD animal model. In this study, 45 Sprague-Dawley rats were divided into three groups. Group 1 (n=15) was received daily peritoneal injection through the catheter connected to the abdominal cavity, using PD solution contain 3.86% D-Glucose. Group 2(n=15) was received daily peritoneal injection of 0.9% physiological saline through a catheter. Group 3 (n=15) was only received sham operation saved as a control. Results showed that WBC counts of peritoneal effluent in G1 group were slightly higher than that of G2 and control group, respectively ($p < 0.05$). However, there is no episode of infection was seen in each group. In addition, there was no significant difference of neutrophils fractions among three groups ($p > 0.05$). H&E and Masson's trichrome staining showed that dramatically increased in thickness of the mesothelium-to-muscle layer of peritoneum exposed to high glucose, compared to G2 and control ($p < 0.01$). These data indicated that a novel rat model of PD with a modified catheter insertion method was established with well, which would be more practical, easily to operate, not too cost and more facilitate to investigate the long-term effects of PD.

Key words

Peritoneal dialysis, rat model, dialysis solution, glucose

Introduction

Over the past 15 years, peritoneal dialysis(PD) has undergone considerable development from a technological point of view(Teitelbaum *et al.*2003). Together with the development, animal models play an essential role in physiological, pathophysiological and clinical aspects of peritoneal dialysis. Since an ideal animal models can adequately simulate the process of PD in humans and provide information for investigating the structure and physiology of peritoneal membrane, as well as the process of peritoneal dialysis, pathophysiology of peritoneal transport, structural changes and local peritoneal defense mechanisms(McIntyre CW *et al.*2007). But there is still no consensus on the ideal experimental model for studying peritoneal dialysis, especially for long-term peritoneal dialysis (Topley N *et al.*2005, Gonzalez-Mateo GT *et al.*2008, Mortier S *et al.*2005).

Recent studies has indicated that there are several factors which affect peritoneum structure, including uremic changes in the internal environment (Plum J *et al.*2001, Williams JD *et al.*2002, Mortier S *et al.*2003, Combet S *et al.*2001), and bioincompatible PD solution during PD process(Obradovic MM *et al.*2000, Zareie M *et al.*2005, De Vriese AS *et al.*2006, Boulanger E *et al.*2007, Kihm LP *et al.*2008). In addition, there are some important technical problems, such as catheter obstruction and high incidence of peritonitis still remain in animal models, which can cause structural and functional changes of peritoneal barrier(Mortier S *et al.*2005, Flessner MF *et al.*2007). It is urgent for us to develop a novel method to overcome these technical problems of PD animal models.

To solve this technical problem above, we established a novel rat model with a modified catheter insertion method, which would be more practical, easily to operate, not too cost and more facilitate to investigate the long-term effects of PD.

Materials and methods

Catheters

The catheter was designed for rat models of PD and had been applied for patent in China (patent number: 200920310638X). The catheter was made by silicone tube, 9cm long and 2 mm in diameter with an iodophor cap on external catheter branch. The iodophor cap can be easily replaced with well. A dacron cuff wrapped the silicone tube and the sutures were fixed on the catheter. There are ten small holes on the intraperitoneal part (1 mm in diameter each) of the tube (Figure 1). The length of catheters can be adjusted according to rat's weight.

Animals

Animal experiments were performed in accordance with the regulation set by the institutional committee for the care and use of laboratory animals, and were approved by the local authorities. Sprague-Dawley rats were housed for 21-28 days on a 12 hours light/dark cycle, and well allowed free access to food and water.

In this study, 45 healthy Sprague-Dawley rats with weighing 200±20g (Shilaite Lab.) were performed. These rats were kept in individual cage and received standard rat's pellets (Veterinary Institute, China) and water. All rats were allowed to adapt to the new living conditions for one week prior to catheter insertion. During the studying period of six weeks, animal's daily behaviors were recorded carefully. The parameters included body mass, body temperature, food intake, Urine volume, infection, antibiotics administration when necessary.

45 Sprague-Dawley rats were divided into three groups. Group one (G1) (n=15) was received daily peritoneal injection through the catheter connected to the abdominal cavity, using peritoneal dialysis solution contain 3.86 %D-Glucose (Baxter China). Group two (G2) (n=15) was received

daily peritoneal injection of 0.9% physiological saline through a catheter. Group three (n=15) was only received sham operation as a control.

Anesthesia procedure

Rats were anesthetized according to an existing protocol which was adapted during the study. Before catheter implantation and sham operation, rats were anesthetized intraperitoneal injection with 0.3ml/100mg chloralhydrate per body mass. This dose of chloralhydrate was sufficient for adequate catheter implantation showing no obvious adverse effects on the rats. The majority of rats were anesthetized in 3 minutes and keep in anesthesia condition for 2 hours.

Catheter implantation

When anesthetized, the rats were shaved on the back of the neck between the ears and scapulae, as well as right back or left back under the arcus costarum. The surgical field was prepared in a standard way. Using an aseptic technique, a 2cm longitudinal incision in the skin of the left or right back, 1cm below the arcus costarum and 1cm lateral to the spinal column was made which named incision 1. A blunt dissection of this area was performed till to the retroperitoneal membrane. Then the catheter tip was inserted into the peritoneal cavity and secured with a purse stitch using suture 4-0 to fix catheter well. Another 1cm longitudinal incision was made in the skin of the posterior neck which on the line between two ears named as incision 2 (shown in fig 2). Before closure of the skin of the back, 10 ml 0.9% physiological saline was administered via the iodophor cap to the catheter to check all possibility of leakage. Then the skin overlying the back was sewed up with single sutures. The external catheter was drawn out from the incision of neck, and the skin was sewed up using suture silk on the catheter, and the tressis vulnus was treated and the skin was sewed with traditional methods. At last, 1ml 0.9% physiological saline containing 5 mg of cefazolin was administered via the iodophor cap to the catheter, followed by 1 ml containing 10 U of heparin continue one week in aim to prevent infection and blockage of catheter (**Figure 2**).

Sham-operation

With the same procedure as mentioned above, the sham-operation group was performed. After these incisions were sewed up without catheter inserting, 5 mg of cefazolin was given by intramuscular injection for one week.

The animal was returned to individual cage and carefully monitored until recovery. Every day the rats were checked the wound and sterilized the external iodophor cap. Each animal was examined for wound dehiscence, iodophor cap breakage, weight loss, or abnormal/lack of movement.

Dialysis procedure

After one week of catheter insertion, either 20 ml of preheated PD solution containing 3.86 % D-glucose or 0.9% physiological saline was instilled into the catheter using 23 needle with syringe. Instillation was continued daily for 6 weeks. To prevent respiratory problems caused by large amounts of fluid, the solution should be injected slowly. In order to avoid catheter blockage, the catheter was heparinized with daily injection of 10 IU heparin in 1ml 0.9% physiological saline after instillation of PD solution. The solutions were drained out on 2, 4, 6 week, respectively.

Cell count and bacterial

After the duration (2, 4, or 6 wk) of daily injections, in G1 group and G2 group, 20 ml of 0.9% physiological saline solution was injected through catheter. After anesthesia, Control group was injected intraperitoneally through 23 needle in inferior belly under sterile condition. After 10min, solution can be drained through catheter in G1 group and G2 group using sterile technique. In

Control group residual peritoneal fluid obtained from an incision was made in the midline of the abdominal wall. After this, the incision was sewed up. The residual peritoneal fluid was collected for cell count. A episodes of infection, defined as a positive dialysate culture and a dialysate white blood cell count $>1000/\text{mm}^3$, were diagnosed (De Vriese AS *et al.*2002, Mortier S *et al.*2004).

Tissue collection

At the end of the study, the rat was euthanized with an overdose of pentobarbital sodium, and three different part of abdominal wall samples were collected, about $2 \times 2 \text{cm}$ in size. The abdominal wall excised and immediately fixed in neutral pH-buffered 4% formalin solution for histological analysis. When abdominal wall was cut by surgical scissors, the movement should be done gently avoiding tough stretch.

Histology and Image Analysis

Tissue was dehydrated, embedded in paraffin, and $5\text{-}\mu\text{m}$ sections were cut. Sections were stained with standard hematoxylin–eosin (H&E) and Masson's trichrome stain method. Peritoneal thickness was quantified in two ways: 1) by measuring the distance from an intact mesothelium to the muscle at five random locations on each slide and 2) by measuring the thickest span of peritoneum on each slide (Flessner MF *et al.*,2006). These measurements were performed by three independent observers with a calibrated optical micrometer and light microscopy (Nikon Eclipse).

Statistical analysis

Statistical analysis of our data was made in Spss 17.0 and Microsoft Office Excel 2003. Results were presented as mean \pm SEM. Statistical significance between multiple groups was tested with the Kruskal-Wallis test. Individual groups were subsequently tested using the Wilcoxon-Mann-Whitney test. A P value of <0.05 was considered significant.

Results

General characteristics of the Rats

Forty -five rats were enrolled in the study. After experiment continued 6 weeks, 5 of 45 rats were quit from this study. Two rats were excluded due to serious infection in G1 group. In addition, one rat since the catheter blocked, one rat was died from operation injury in group 2. In control group, one rat died from anesthesia. On the other hand, at the end of the study, white biofilms were found on the catheters surface in 3 rats but it is not obstructed completely.

There was no significant difference among three groups in initial weight and weight changes. All of the rats gained weight during the experimental period. Average weight gains in each group as shown in table 1.

WBC count of peritoneal effluent

As shown in figure 3, on the week 2, white blood cell(WBC) counts of peritoneal effluent in G1 group were a little higher than that of control group and G2 group, respectively, but there are not statistically significance ($p > 0.05$). WBC counts in G1 group were higher than G2 group and Control group in week4 and 6, respectively($*p < 0.01$). However, there is no episode of infection was seen in each group. In addition, there was no significant difference of neutrophils fractions among three groups ($p > 0.05$).

Histological change

As shown in figure 4, Hematoxylin–eosin (H&E) and Masson's trichrome Staining showed

that significant changes in thickness of the mesothelium-to-muscle layer of peritoneum exposed to high glucose compared to G2 and control. The mean thickness of submesothelial compact zone for G1 group was dramatically increased compared to that of G2 and Control group ($*p < 0.05$). There is no difference of submesothelial thickness between G2 and Control group ($p > 0.05$).

Discussion

Animal models play a key role in understanding the function and structure of peritoneal tissues alteration during peritoneal dialysis. The function damage and structural change of peritoneal membrane are associated with the uremic changes and long-term exposure to PD fluid. Beside these factors above, it was demonstrated that silicone catheter widely used peritoneal cavity may induce inflammatory response on peritoneum (Flessner MF *et al.*2007). As reported, a traditional PD animal model using a permanent catheter tunneled from the peritoneal cavity connecting a subcutaneous chamber has some disadvantages, such as difficult of drainage of the dialysate and need anesthesia every time when dialysis solution was administered (Mortier S *et al.*2004, Flessner MF *et al.*2006). In addition, the use of anesthetic can influence peritoneal permeability and kinetics of peritoneal transport by its effect on lymph drainage (Tran L *et al.*1993). On the other hand, intraperitoneal injection directly used in various PD model can cause infection, trauma to peritoneal tissue, or bowel perforation due to repeated punctures (Gonzalez-Mateo GT *et al.*2008, Flessner MF *et al.*2007, Peng WX *et al.*2000).

Since traditional peritoneal catheters caused several common complications, including infection, catheter loss and damage, long external branch irritating the animals, which let us develop a new non-uremic rat model of PD with catheter, it will adequately imitate the process of PD patient, and provide an important information of studying the structure and physiology of peritoneal membrane in PD.

In this experiment, we modified the catheter insertion method and constructed a new rat model, which showed no catheter pulling out and low infection (Figure 3). Because the rate of the catheter pull out was very high without additional measures in traditional PD animal model, the added suture fixed on catheter was used to avoid catheter dropping off (Figure 2). The dacron cuff on catheter promoted tissue growth then can prevent bacterial spread through catheter' surface. The iodophor cap used for injection and drainage was more convenient than subcutaneous chamber for subcutaneous injection.

Other serious technical failures in animal models of long-time PD are frequent obstruction of peritoneal access and concomitant infection. The initial step in the formation of catheter-associated biofilm is adherence of free-floating, or planktonic, organisms to the catheter surface. This occurs through cell wall-associated adhesions, such as microbial surface components recognizing adhesive matrix molecules, and is facilitated by the deposition of a conditioning film of fibrin and fibronectin on the catheter surface (Trautner BW *et al.*2004, Aslam S *et al.*2008). To avoid biofilm, heparin was used to minimize the formation of biofilm. Because heparin can inhibit thrombin and fibrin formation and by virtue of preventing thrombin generation and activity, can thus inhibit platelet activation (Niers TM *et al.*2007). In this observation, only 3 of 40 PD rat were found that the earliest biofilm formed in three days after catheter implantation, which consensus with described previously (Zunic-Bozinovski S *et al.*2008).

Catheters inserted through the abdominal wall into the cavity and caused a significant change on the inflammatory process in the peritoneum (Flessner MF *et al.*2007){Flessner, 2007 #16}. To

reduce the direct irritation on peritoneal membrane, in this study the catheter was inserted through the back wall (shown in figure 2). As result, it showed minor structural alteration in the peritoneum, we therefore hypothesized that the catheter implanted through back wall rather than through abdominal wall may minimize effects on peritoneum.

The thickness of submesothelial tissue notably increased as detected by HE and masson staining in G1 rat PD model, which instilled with 3.86% D-glucose by the modified catheter insertion method. By the quantitatively analysis, the thickness of peritoneal tissues was significantly increased in G1 group compared to G2 and control (shown in fig 4) suggested a long-time PD animal model was constructed successfully.

In this study, we found that the surgical treatment on rats PD model was easily performed and the wound caused by surgery is minor. Complications such as peritonitis, catheter damage, catheter obstruction can be negligible with the method introduced in this study. It indicated that this new non-uremic rat model with modified catheter insertion method can be used to analyze the effects of PD on rat peritoneum with minor structural alterations caused by catheter compared to traditional method of PD animal model.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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FIGURE LEGENDS

Table 1: Rats general characteristics (Values are means \pm SEM)

Forty -five rats were enrolled in the study with fifth rats in each group. During the follow up period, 5 rats were quit from this study. Two rats were excluded due to serious infection in G1 group. In addition, in G2 group, one rat was recorded with catheter obstruction and one rat was died from operation injury. In control group, one rat died from anesthesia.

The initial weight of each group was about 180g. All of the rats gained weight during the experimental period.

Figure1: Schematic of the catheter.

The catheter was designed for rat models of PD and had been applied for patent in China (patent number: 200920310638X). (For detailed explanation see the text).

Figure2: Schematic of the Model and photograph of the Successful model.

One 1cm longitudinal incision was made in the skin of the posterior neck which on the line between two ears, and the other 2cm longitudinal incision was made in the skin of the left or right back, 1cm below the arcus costarum and 1cm lateral to the spinal column. The right figure is the photograph of the successful model. (For detailed explanation of the surgical procedure of catheter placement see the text)

Figure 3: the total white blood cell (WBC) count for each group and the fraction of neutrophils

in each WBC

Total white blood cells count per mm³ of the peritoneal fluid *versus* the duration in weeks of daily injection. Mean values are the time-averaged means of each injection group. On the 2 week, the WBC count of dialysis solution in G1 group was higher than the Control group and the G2 group, but the difference was not statistically significant ($p > 0.05$). The WBC count in G1 group was higher than Control group and G2 group in 4 and 6 week, the difference was statistically significant ($*p < 0.01$), there were no significant difference between the Control group and the G2 group ($p > 0.05$).

Fractions of neutrophils were greater in the G1 group than control group and G2 group, but the differences were not statistically significant. ($p > 0.05$)

Figure 4: thickness of peritoneum

Peritoneal thickness (μm) *versus* duration of injection. The mean thickness for the G1 group was significantly different from that for the control group and G2 group ($*p < 0.01$). Open bars are derived from five random measurements in each section, and filled bars are derived from points of maximum thickness.

Comparison of peritoneal thickness (μm). (HE \times 200). Bar = 100 μm .

Comparison of peritoneal thickness (μm). (MASSON \times 200) \times 200. Bar = 100 μm .

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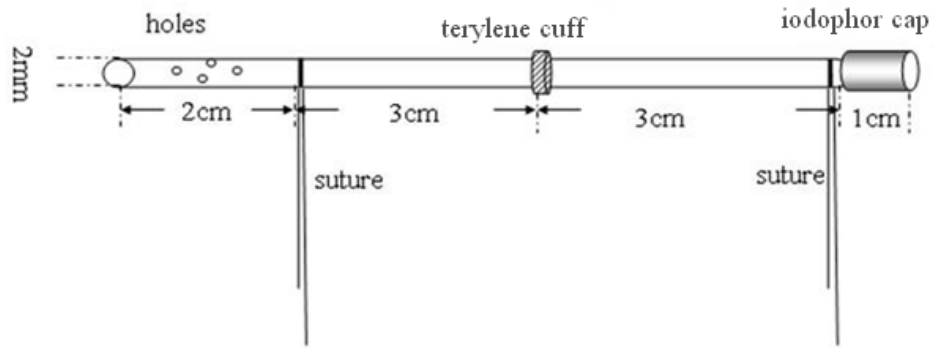
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Table 1:Rats general characteristics (Values are means \pm SEM)

| Group | Total number | Exclude number | Analyzed number | Weight(g) | | | |
|-------|--------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | | | 0week | 2weeks | 4weeks | 6weeks |
| G1 | 15 | 2 | 13 | 187.3 \pm 2.7 | 234.1 \pm 3.7 | 268 \pm 5.6 | 289.9 \pm 6.2 |
| G2 | 15 | 2 | 13 | 184.6 \pm 5.3 | 229.4 \pm 6.1 | 267.8 \pm 3.1 | 292.9 \pm 2.5 |
| CON | 15 | 1 | 14 | 183.8 \pm 4.5 | 236.9 \pm 5.1 | 271 \pm 6.3 | 296 \pm 5.8 |

Figure1: Schematic of the catheter.



Patent number: 200920310638X

Figure2: Schematic of the Model and photograph of the Successful model

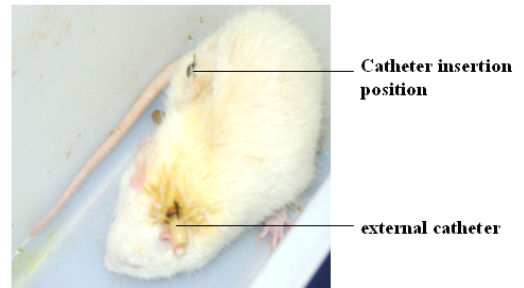
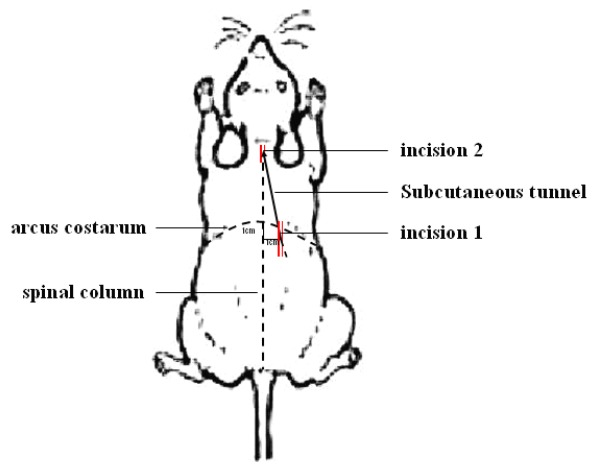


Figure 3: the total white blood cell (WBC) count for each group and the fraction of neutrophils in each WBC

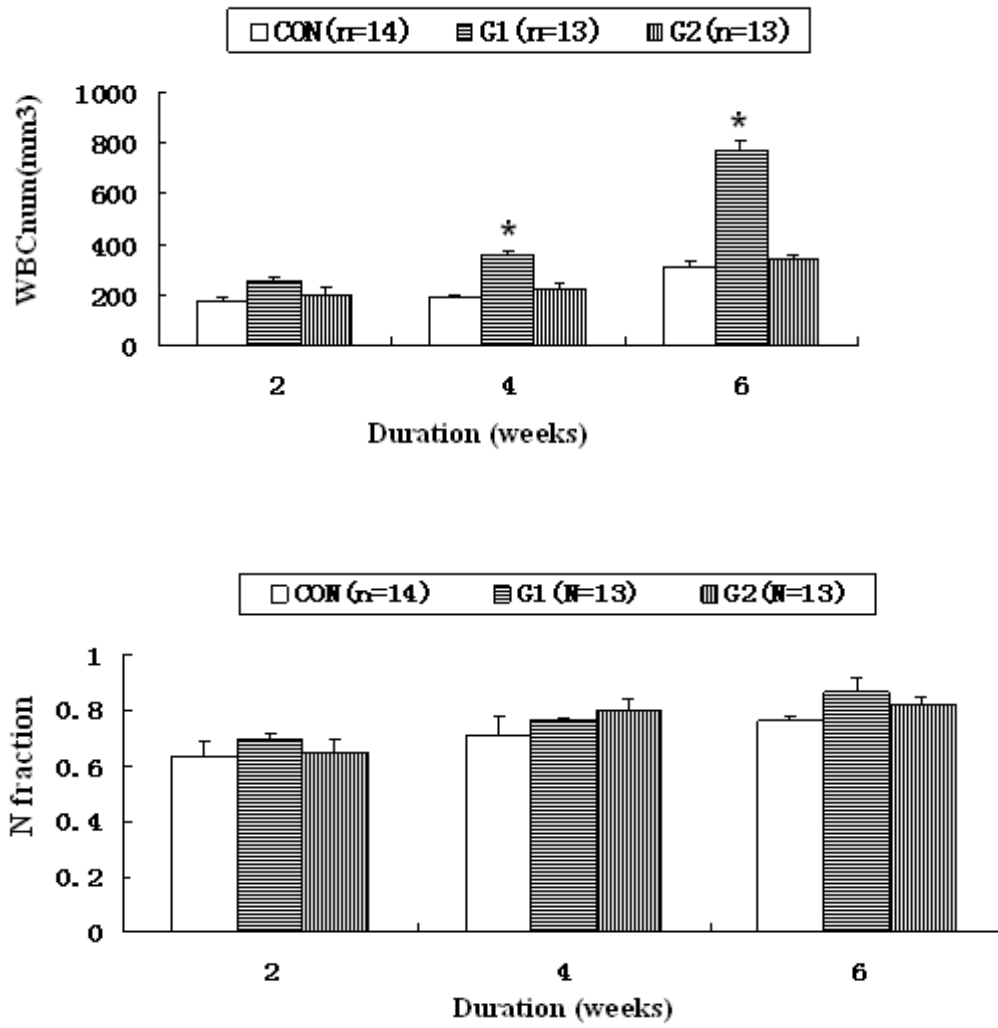
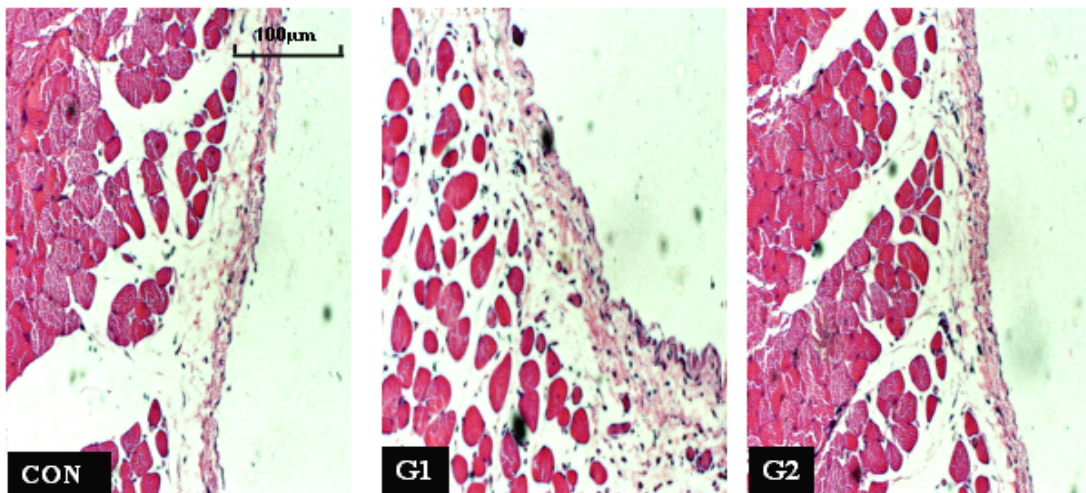
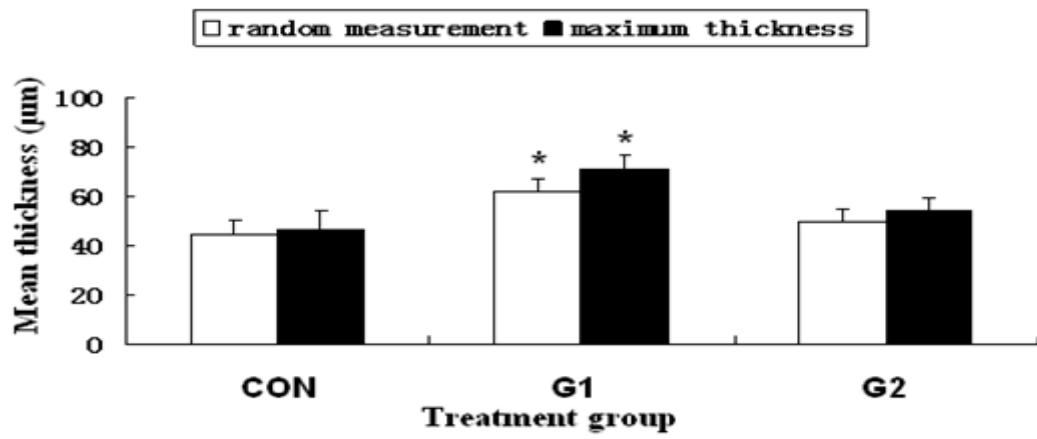
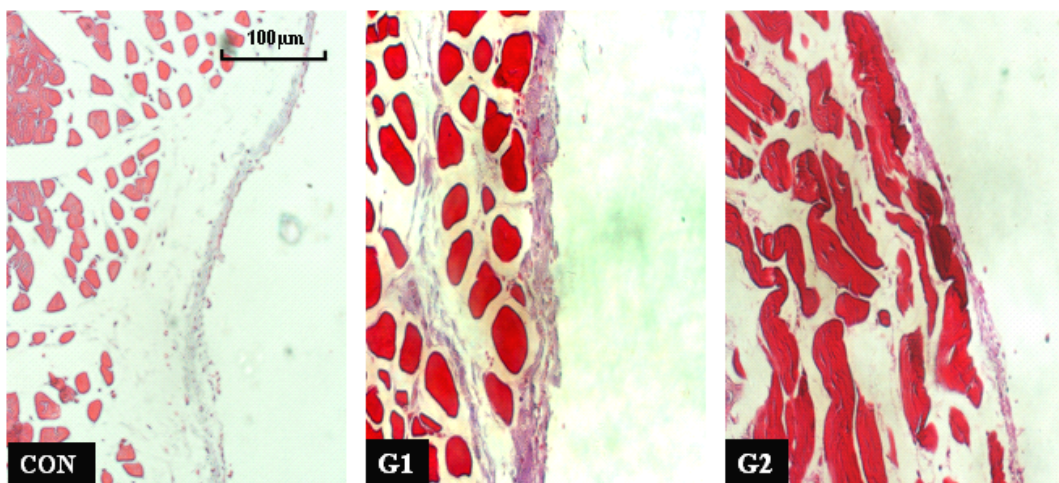


Figure 4: thickness of peritoneum



HE × 200



MASSON × 200