EFFECTS OF SULODEXIDE IN THE PREVENTION OF PERITONEAL MEMBRANE CHANGES I N CONTINUOUS AMBULATORY PERITONEAL DIALYSIS PATIENTS

Miss Panida Ditsawanon

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Pharmaceutical Care
Department of Pharmacy Practice
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2019

ผลของยาซูโลเดกไซด์ในการป้องกันการเปลี่ยนแปลงเยื่อบุช่องท้องในผู้ป่วยที่ได้รับการล้างไตทางช่องท้องแบบต่อเนื่อง

น.ส.ปณิดา ดิศวนนท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
สาขาวิชาการบริบาลทางเภสัชกรรม ภาควิชาเภสัชกรรมปฏิบัติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2562

Thesis Title	EFFECTS OF SULODEXIDE IN THE PREVENTION OF PERITONEAL MEMBRANE CHANGES IN CONTINUOUS AMBULATORY PERITONEAL DIALYSIS PATIENTS
By	Miss Panida Ditsawanon
Field of Study	Pharmaceutical Care
Thesis Advisor	Professor PORNANONG ARAMWIT, Ph.D.
Thesis Co Advisor	Senior Colonel Ouppatham Supasyndh, M.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the Faculty of Pharmaceutical Sciences

(Assistant Professor Rungpetch Sakulbumrungsil, Ph.D.)

DISSERTATION COMMITTEE

..... Chairman

(Associate Professor Thitima Pengsuparp, Ph.D.)

..... Thesis Advisor

(Professor PORNANONG ARAMWIT, Ph.D.)

..... Thesis Co-Advisor

(Senior Colonel Ouppatham Supasyndh, M.D.)

..... Examiner

(Assistant Professor Thitima Wattanavijitkul, Ph.D.)

..... Examiner

(Assistant Professor CHANKIT PUTTILERPONG, Ph.D.)

..... External Examiner

(Assistant Professor Sumate Ampawong, Ph.D.)

ปณิดา ดิศวนนท์ : ผลของยาซูโลเดกไซด์ในการป้องกันการเปลี่ยนแปลงเยื่อบุช่องท้องในผู้ป่วยที่ได้รับการล้างไตทางช่องท้องแบบต่อเนื่อง. (
EFFECTS OF SULODEXIDE IN THE PREVENTION OF PERITONEAL MEMBRANE CHANGES IN CON TINUOUS AMBULATORY PERITONEAL DIALYSIS PATIENTS) อ.ที่ปรึกษาหลัก : ศ. ภญ. ดร.พรอนงค์ อร่ามวิทย์, อ.ที่ปรึกษาร่วม : พ.อ.(พิเศษ) นพ.อุปถัมภ์ ศุภสินธุ์

จุดประสงค์ของการศึกษาที่มีกลุ่มควบคุมนี้คือเพื่อศึกษาผลของยา sulodexide ในการป้องกันการเปลี่ยนแปลงเยื่อบุช่องท้องในผู้ป่วยที่ได้รับการล้างไตทางช่องท้องแบบต่อเนื่อง โดยประเมินจากการเปลี่ยนแปลงของตัวชี้วัดทางชีวภาพในน้ำยาล้างไต การเปลี่ยนแปลงรูปร่างของเซลล์เยื่อบุช่องท้อง การแลกเปลี่ยนสารของเยื่อบุช่องท้อง และความปลอดภัยในผู้ป่วย การศึกษานี้มีผู้ป่วยเข้าร่วมการศึกษาทั้งหมด 66 คน โดยผู้ป่วยถูกสุ่มให้ได้รับ sulodexide 100 mg/วัน โดยการรับประทานหรือได้รับยาหลอก ผู้วิจัยได้ทำการทดสอบ peritoneal equilibrium test เพื่อประเมินความสามารถในการแลกเปลี่ยนสารของเยื่อบุช่องท้อง มีการวัดระดับความเข้มข้นของ CA125 IL-6 และ VEGF ในน้ำยาล้างไตของผู้ป่วยโดยวิธี ELISA ทั้งก่อนเริ่มยาและหลังจากได้รับยานาน 3 เดือน และได้ทำการเพาะเลี้ยงเซลล์เยื่อบุช่องท้องที่ได้จากน้ำยาล้างไตของผู้ป่วยเพื่อประเมินการเปลี่ยนแปลงรูปร่างของเซลล์

มีผู้ป่วยเข้าร่วมจนจบการศึกษาเป็นจำนวน 61 คน หลังจากครบระยะเวลาให้ยา 3 เดือน พบว่ากลุ่มที่ได้รับยา sulodexide มีค่า D/P creatinine ลดลงมากกว่ากลุ่มที่ได้รับยาหลอก (p-value = 0.04) อย่างไรก็ตามไม่พบความแตกต่างของค่า D/D0 glucose ระหว่างทั้ง 2 กลุ่ม สำหรับค่า ultrafiltration volume พบว่ากลุ่มที่ได้รับยา sulodexide มีค่าสูงกว่าเมื่อเทียบกับกลุ่มที่ได้รับยาหลอก (p-value = 0.01) และในด้านตัวชี้วัดทางชีวภาพในน้ำยาล้างไตพบว่าหลังได้รับยาไม่มีความแตกต่างของระดับ CA125 จากค่าเริ่มต้นในกลุ่มที่ได้รับยา sulodexide ในขณะที่กลุ่มที่ได้รับยาหลอกมีระดับ CA125 ลดลงจากค่าเริ่มต้น (p-value = 0.03) อย่างไรก็ตามไม่พบความแตกต่างของระดับ CA125 ระหว่างทั้ง 2 กลุ่ม และเมื่อเปรียบเทียบระดับ IL-6 พบว่าในกลุ่มที่ได้รับยาหลอกมีค่า IL-6 เพิ่มสูงขึ้นจากค่าเริ่มต้น (p-value < 0.01) แต่ไม่พบความแตกต่างที่ก่อนและหลังจากได้รับยาในกลุ่มที่ได้รับยา sulodexide เมื่อเปรียบเทียบระหว่างทั้ง 2 กลุ่ม พบว่ากลุ่มที่ได้รับยาหลอกมีระดับ IL-6 สูงกว่ากลุ่มที่ได้รับยา sulodexide อย่างมีนัยสำคัญ (p-value = 0.03) สำหรับระดับความเข้มข้นของ VEGF นั้นพบว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญทั้งภายในกลุ่มทั้ง 2 กลุ่ม และระหว่างกลุ่มที่ได้รับยา sulodexide และกลุ่มที่ได้รับยาหลอก นอกจากนี้ยังไม่พบความแตกต่างอย่างมีนัยสำคัญของจำนวนการเกิดเหตุการณ์ไม่พึงประสงค์ระหว่างที่ได้รับยาของทั้ง 2 กลุ่ม

จากผลการศึกษาทั้งหมดนั้นสรุปได้ว่าการได้รับยา sulodexide โดยการรับประทาน อาจมีผลในการป้องกันการเปลี่ยนแปลงเยื่อบุช่องท้องในผู้ป่วยที่ได้รับการล้างไตทางช่องท้องได้ และอาจสามาช่วยชะลอการเปลี่ยนแปลงของเยื่อบุช่องได้

สาขาวิชา	การบริบาลทางเภสัชกรรม	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5576553133 : MAJOR PHARMACEUTICAL CARE

KEYWORD: sulodexide, peritoneal dialysis, peritoneal membrane, biomarker

Panida Ditsawanon :

EFFECTS OF SULODEXIDE IN THE PREVENTION OF PERITONEAL MEMBRANE CHANGES IN CON TINUOUS AMBULATORY PERITONEAL DIALYSIS PATIENTS. Advisor: Prof. PORNANONG ARAMWIT, Ph.D. Co-advisor: Sr COL. Ouppatham Supasyndh, M.D.

The objective of this placebo-controlled clinical study is to determine the effect of sulodexide for the prevention of peritoneal membrane change in PD patients by evaluating dialysate biomarkers of peritoneal membrane change, phenotypes of peritoneal mesothelial cells, peritoneal membrane transports and safety. A total of 66 patients were included in this randomized control trial study. Patients were randomly assigned to receive either 50 mg of sulodexide or placebo by oral 2 times daily. PET was performed to evaluate peritoneal transport function. Dialysate CA125, IL-6 and VEGF concentration were also measured at baseline and after treatment by ELISA. Peritoneal mesothelial cell culture from dialysate effluent was done to evaluate EMT.

Overall, 61 patients completed the 3-month study. After the treatment period, there was a significantly lower D/P creatinine in the sulodexide group than in the placebo group (p-value = 0.04). However, no difference in D/D0 glucose was observed between two groups. For ultrafiltration volume, there was a significantly higher volume in the sulodexide group when compared to the placebo group (p-value = 0.01). Patients in the sulodexide group had no difference change from baseline in CA125 concentration while there was a significantly lower CA125 level in the placebo group (p-value = 0.03). However, no significant difference in CA125 was found between the two groups. For IL-6 , a significantly higher level was found within the placebo group after the treatment period (p-value < 0.01) while there was no difference change within the sulodexide group. When compared between groups, a significantly higher IL-6 level was found in the placebo group than those in the sulodexide group (p-value = 0.03). No difference was observed for VEGF changes both within and between two groups of patients. In conclusion from overall results in this study, the administration of sulodexide has a potentially beneficial effect in the prevention of peritoneal membrane damage in CAPD patients. Sulodexide may be used to slow the progression of peritoneal membrane change.

Field of Study: Pharmaceutical Care Student's Signature .....

Academic Year: 2019 Advisor's Signature .....

Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my thesis advisor, Professor Pornanong Aramwit, Pharm.D., Ph.D. of the Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University and my thesis co-advisor, Colonel Ouppatham Supasyndh, M.D. of the Division of Nephrology, Department of Medicine, Phramongkutklao Hospital and College of Medicine, for their valuable advice and guidance and all their support and kindness throughout the study.

I am very thankful to the members of my thesis committee for their valuable suggestions and comments.

I gratefully acknowledge the financial support for my study from the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program. I also gratefully acknowledge all the support from Faculty of Pharmaceutical Sciences, Chulalongkorn University. I would like express my deep appreciation to Piyatida Cheungsaman, M.D. of Banphaeo Dialysis Group (Bangkok), Banphaeo Hospital, for facilitate with the patients' recruitment at Banphaeo Hospital. I would like to express my appreciation to all the nurses at Division of Nephrology, Phramongkutklao Hospital for support their support during the study.

Moreover, I would like to express my deepest gratitude to all the patients who participated in this study and all of those whose names have not been mentioned in recognition of their support in whatever way.

Finally, I am deeply thankful to my family for their encouragement and support throughout my life.

Panida Ditsawanon

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

ACEI	=	Angiotensin converting enzyme inhibitors
AGEs	=	Advanced glycation end products
ARB	=	Angiotensin receptor blockers
CA	=	Cancer antigen
CAPD	=	Continuous ambulatory peritoneal dialysis patients
CTGF	=	Connective tissue growth factor
D/D0	=	Dialysate at x minutes /initial dialysate ratio of glucose
D/P	=	Dialysate over plasma ratio
ECM	=	Extracellular matrix
ELISA	=	Enzyme-linked immunosorbent assay
EMT	=	Epithelial-mesenchymal transition
EPS	=	Encapsulating peritoneal sclerosis
ESRD	=	End-stage renal disease
GDPs	=	Glucose degradation products
HD	=	Hemodialysis
IL	=	Interleukin
LMWH	=	Low molecular weight heparins
MCP	=	Monocyte chemoattractant protein
MMP	=	matrix metalloproteinases
PET	=	Peritoneal equilibrium test
PD	=	Peritoneal dialysis
RAAS	=	Renin-angiotensin-aldosterone system
RRF	=	Residual renal function

TGF	=	Transforming growth factor
TIMP	=	Tissue inhibitors of metalloproteinases
UF	=	Ultrafiltration
VEGF	=	Vascular endothelial growth factor

CHAPTER I

INTRODUCTION

1.1 Rationale and Background

End-stage renal disease (ESRD) is a public health problem worldwide. Peritoneal dialysis (PD) is one of the established options for renal replacement therapy used by approximately 11% of patients with ESRD around the world [1]. In 2008, Thai government implemented a “PD first” policy to Thai ESRD patients under the Universal Health-Care Coverage (UC) scheme, encouraging the use of PD as an initial treatment of patients with ESRD [2]. In Thailand, the prevalence of PD in 2015 was 369 per million population and the number of PD patients rose from 2009 which was only 81 per million population [3].

Diffusion and convection are the mechanisms involved in the transport of solutes during PD. Diffusion through a peritoneal membrane takes place when a concentration gradient between blood flow and dialysis solution is present. Convection takes place through glucose which is an osmotic agent in dialysis solution. These mechanisms cause the removal of waste and extra fluid from the body [4]. However, in patients undergoing long-term PD, alterations in the structure and transport function of the peritoneal membrane can occur and lead to the reduction of the peritoneal membrane’s capacity to remove salt, water, and uremic toxins. Eventually, these alterations can result in ultrafiltration failure and peritoneal fibrosis [5, 6].

When PD can no longer be used in ESRD patients, they have to transfer from PD to hemodialysis (HD) which is more complicated and higher costs. The prevalence of peritoneal membrane dysfunction as a cause of PD drop-out has been reported to be

between 1.7% and 13.7% [7], therefore it is important to prevent peritoneal membrane dysfunction in PD patients in order to prolong the time before switching to HD. One of the preventive options is the use of glycosaminoglycans. Heparin belongs to the glycosaminoglycans family. There are some data that heparin has shown beneficial effects in the reduction of the peritoneal membrane alteration besides its anticoagulant effect [8, 9]. However, heparin use has some limitations due to its serious side effects, such as bleeding tendency and thrombocytopenia, and inconvenient administration methods which can only be administered subcutaneously and intraperitoneally [10, 11]. Thus, Sulodexide is considered to be an alternative option in glycosaminoglycans family. Sulodexide, which is a mixture of glycosaminoglycan consisting of fast moving heparin 80% and dermatan sulfate 20% [12], can be administered orally and appears to have fewer serious side effects than heparin [11].

Sulodexide has been used as an antithrombotic drug and to reduce proteinuria in diabetic nephropathy. Moreover, it has been reported to decrease peritoneal membrane dysfunction. Previous studies in animal models demonstrated that sulodexide administered intraperitoneally, subcutaneously and orally could decrease peritoneal membrane transformation [13-15]. In several uncontrolled clinical studies, they found that sulodexide administered orally and intraperitoneally could also decrease peritoneal membrane dysfunction [16, 17]. There has not been any good design randomized placebo-controlled clinical study that explores the efficacy and safety of orally administered sulodexide in preventing peritoneal membrane dysfunction and there is no study that investigates in a molecular level. Therefore, we conducted a different study design, which is a randomized placebo-controlled study, in order to verify the advantage of sulodexide in peritoneal membrane preservation in CAPD patients. The expected benefit of this study is that we can explore the use of sulodexide in slowing the progression of peritoneal membrane change and delaying the PD drop-out in CAPD patients. The objective of this placebo-controlled clinical study is to determine the effect of sulodexide for the prevention of peritoneal membrane change in PD patients by

evaluating dialysate biomarkers of peritoneal membrane change, phenotypes of peritoneal mesothelial cells, peritoneal membrane transports and safety.

1.2 Hypotheses

1.2.1 Sulodexide-treated group has an increase in dialysate CA125, a decrease in dialysate IL-6 and VEGF levels as compared to the placebo group.

1.2.2 Sulodexide-treated group has better or no change from baseline in peritoneal membrane transport as compared to the placebo group.

1.2.3 Sulodexide-treated group has no difference in adverse event rates as compared to the placebo group.

1.3 Objectives

1.3.1 Primary objective

To determine the effects of sulodexide on dialysate CA125, IL-6, VEGF levels in CAPD patients who receive Sulodexide compared to placebo.

1.3.2 Secondary objectives

- To determine the effects of sulodexide on peritoneal membrane transport in CAPD patients who receive sulodexide compared to placebo.
- To determine the effects of sulodexide on adverse event rates in CAPD patients who receive sulodexide compared to placebo.

1.4 Scopes

1.4.1 Peritoneal fluid, blood and urine samples from CAPD patients were collected. Peritoneal membrane function test and dialysate CA125, IL-6, VEGF levels were measured to examine the effects of Sulodexide on

peritoneal membrane function and dialysate biomarkers between two groups.

1.4.2 Adverse event rates were collected to assess the safety of Sulodexide in CAPD patients.

1.5 Expected Outcomes

The results of this study may verify the advantage of sulodexide in peritoneal membrane preservation in CAPD patients. Sulodexide can be used to slow the progression of peritoneal membrane change and delay the switching from PD to HD. We can also find out whether it is possible to be a new indication of this drug.

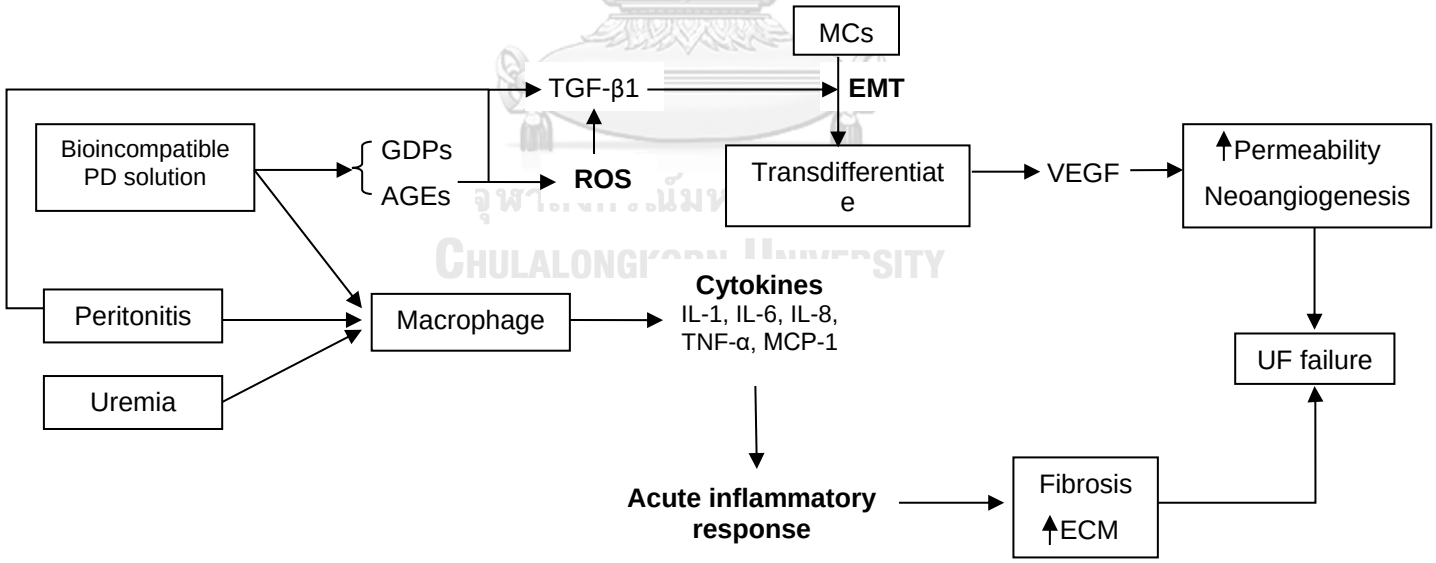
CHAPTER II

LITERATURE REVIEWS

2.1 Peritoneal membrane changes in peritoneal dialysis patients

The success of PD depends on maintaining the structural and functional integrity of the peritoneal membrane in removing salt, water, and waste products. Structural and functional changes of the peritoneal membrane are associated with long-term PD. (Figure 1)

Figure 1 Pathways and factors contributing to peritoneal structural and functional changes in PD patients



GDPs = glucose degradation products; AGEs = advanced glycation end-products; TGF- β1= transforming growth factor; ROS = reactive oxygen species; MCs = mesothelial cells; IL = interleukin; TNF- α = tumor necrosis factor-α; MCP-1 = monocyte chemoattractant protein; VEGF = vascular endothelial growth factor; ECM = extracellular matrix

2.1.1 Peritoneal structural changes

Peritoneal membrane is a semi-permeable heteroporous structure that is formed of 4 major systems including [18]

1. Mesothelial cells monolayer
2. Submesothelium interstitial tissue
3. Capillary system
4. Lymphatic system

During PD, these systems are exposed to the dialysis solutions, peritonitis, uremia, and chronic inflammation. These factors initiate the activation of peritoneal cells, such as macrophages, mast cells, mesothelial cells, fibroblasts, and endothelial cells. Growth factors and cytokines are subsequently released. Overall, these factors lead to the loss of mesothelial cells. Because of the disruption of the balance between matrix synthesis and degradation, which can be termed a pathological wound healing process that exceeds the physiological repair process, the accumulation of matrix proteins within the submesothelial layer can occur and leads to an increase in the thickness of the submesothelial layer. It also causes vasculopathy, which leads to neoangiogenesis. In the case of long-term exposure to insult, there is an accumulation of matrix proteins within the submesothelial layer because of disruption to the balance between matrix synthesis and degradation; which can be termed a pathological wound healing process that exceeds the physiological repair process [5-7, 10]. Severe stage of peritoneal membrane damage called Encapsulating Peritoneal Sclerosis (EPS). It is a fatal manifestation; a persistent or recurrent intestinal obstruction, with or without inflammatory parameters of peritoneal thickening, sclerosis, calcification, and encapsulation [5, 6, 19, 20].

In addition to the change in a number of cells from the loss of mesothelial cells, there is a morphological change of mesothelial cells to a more fibroblastoid phenotype. This switch in phenotype from epithelioid to fibroblastoid, which has mesenchymal cell

properties, is called Epithelial to Mesenchymal Transition (EMT). The fibroblastoid phenotype can move into the submesothelial compact zone, where they may contribute directly to the fibrotic and angiogenic processes [21].

Plum J, et al [22] studied histopathological changes of the peritoneal membrane between PD patients, uremic patients before onset of PD and normal patients, they found that an increase of the submesothelial fibrous tissue was a common finding during PD. The increased thickness of the submesothelial layer was showed in uremic patients as compared with normal patients, and more increased in PD patients. Patients on PD also had an increased density of small vessels and capillaries in submesothelial layer. The wall/lumen index of vessels was increased indicating vascular sclerosis. The mesothelial cell layer was rather well preserved in normal patients. Changes in the mesothelial ultrastructure associated with a loss of microvilli and hyperplasia of the rough endoplasmic reticulum were shown.

Yanez-Mo M, et al [23] demonstrated that mesothelial cells isolated from peritoneal effluents in PD patients undergo a transition from an epithelial phenotype to a mesenchymal phenotype with a progressive loss of epithelial morphology and a decrease in the expression of epithelial phenotype markers.

2.1.2 Peritoneal functional changes [21-24]

The changes of peritoneal membrane function consist of peritoneal hyperpermeability of glucose and uremic toxins, which is one factor that influences the dialysis adequacy. Afterward, the decrease in glucose in the dialysis solution can result in ultrafiltration failure, which refers to the inability to achieve volume homeostasis, and subsequently volume overload. Finally, these changes lead to mortality risk or a requirement to transfer from PD to HD.

Peritoneal membrane solute and water transport properties in PD patients are usually assessed by the Peritoneal Equilibrium Test (PET). This test evaluates low molecular weight solute transfer and ultrafiltration capacity.

The major changes include an increase in small solute transport rate and a decrease in ultrafiltration. There is a negative association between solute transport and ultrafiltration capacity, which is a higher small solute transport, a lower ultrafiltration capacity. There was a Stoke cohort study found that solute transport increased in the 6 months after starting PD, and continue throughout the course of treatment. This causes increased transport, which will lead to the more rapid absorption of glucose with the abolition of the osmotic gradient and reduced ultrafiltration. Consequently, reduced ultrafiltration will lead to fluid overload.

The factors believed to cause these alterations with time on PD include repeated episodes of peritonitis and long-term exposure to bioincompatible dialysates. There are longitudinal studies reporting that increased use of hypertonic glucose results in peritoneal membrane function changes.

The CANUSA study in America and Canada found that patients with different transport status had different patient and technique survival. A worse patient and technique survival was associated with high transport status. Consistent with the ANZDATA registry, they reported that a high transport status was associated with increased mortality and technique failure.

Nowadays, the morphological cause for a progressive increase in small solute transport is unclear. It is probably because of the alteration of endothelial peritoneal lining capillaries [25] or mesothelial cells changes that lead to peritoneal fibrosis and neoangiogenesis.

2.2 Factors contributing to peritoneal membrane changes in peritoneal dialysis patients

Several important factors can contribute to peritoneal membrane remodeling such as uremia, peritonitis and particularly the exposure of the membrane to bioincompatible dialysis solution.

2.2.1 Uremia

In uremic patients, there are significant increases in Nitric Oxide (NO), Advanced Glycation End Products (AGEs), Vascular Endothelial Growth Factor (VEGF) and inflammatory cytokines such as Interleukin (IL)-1β and Tumor Necrosis Factor-alpha (TNF-α). These factors are known to modulate the structure and function of the peritoneal membrane [26]. There is no exactly known mechanism, but there is a hypothesis that functional changes in peritoneal membrane correlate with structural changes. Williams, et al [27] studied the peritoneal biopsy in uremic patients, they found that uremia induced the thickening of the submesothelial compact zone almost three times than normal individuals. Moreover, neovascularization was observed in the uremia group.

2.2.2 Peritonitis

Peritonitis can contribute to peritoneal changes by inducing mesothelial damage, massive inflammatory responses and increased vascularization of peritoneal tissue, leading to impaired membrane function. NO, proinflammatory cytokines (TNF, IL-1, and IL-6) and prostaglandins are considered to be the inducer of peritoneal membrane injury [5]. Davies, et al [28] performed a study of the effect of peritonitis on membrane function by repeating PETs after every episode of infections. It showed that single episodes with a moderate inflammatory response (dialysate leukocyte counts less

than 2,000 cells/mL.) had no effect on membrane function. Recently, there is a study demonstrated that the changes in membrane function associated with peritonitis with time on therapy. Therefore, peritonitis can augment the membrane function change over time, but it is not the major determinant [29].

2.2.3 Bioincompatible dialysis solution

In healthy individuals, the peritoneal cavity contains minimal amounts of fluid, the composition of which is similar to that of plasma. In PD patients, standard or conventional PD solutions are commonly used. They have to repeat intraperitoneal infusion of dialysis solution which has a different composition from plasma and is bioincompatible with mesothelial cells, the peritoneal membrane, and inflammatory cells. Conventional PD solutions are bioincompatible because of their characteristics, which are as follows [30]:

1. Hypertonicity (osmolarity 358 - 510 mOsm/kg)
2. High concentrations of glucose (15 - 42.5 g/L) to induce transperitoneal ultrafiltration of water.
3. High concentrations of lactate (35 - 40 mmol/L) in order to maintain a low pH (approximately 5.3) that is induced artificially to avoid caramelization during heat sterilization.

Glucose

Nowadays, glucose is used as a major osmotic agent in PD solutions. The peritoneal membrane will absorb 75% of glucose in 6-hour dwell time [31]. Therefore, the peritoneal membrane in this group of patients has similar pathophysiology to diabetic patients, which neovascularization can be observed. There is a study found that glucose can induce the synthesis of VEGF and Transforming Growth Factor- β1 (TGF-β1) [32]. VEGF is a growth factor that has an important role in neoangiogenesis in

diabetic patients, while TGF- β1 has an important role in extracellular matrix accumulation and fibrosis. Moreover, glucose is associated with the balance of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Metalloproteinases (TIMPs), which control matrix synthesis and degradation in various tissue. Glucose can induce TIMPs synthesis. As a result of these effects, patients could have high peritoneal transport status because of an increase in vascular surface area and permeability [26].

Glucose Degradation Products (GDPs) [26]

GDPs is a derivative generated during the heat sterilization of the PD solution. GDPs inhibit proliferation and cause necrosis in several *in vitro* cellular systems, so they interfere with basal cellular functions. For example, the toxic effects of these components on the viability and function of peritoneal leukocytes, fibroblasts, and mesothelial cells. GDPs also enhance local production of VEGF and thus contribute to peritoneal neoangiogenesis. The presence of GDP seemed to be important factor for these changes in the peritoneal microcirculation. It was considered that ultrafiltration capacity may be decreased by chronic capillary recruitment, due to an increase in the effective vascular surface area.

Advanced Glycation End Products (AGEs)

Glucose degrades during heat sterilization into GDPs, which consists of a variety of Reactive Carbonyl Compounds (RCO). These RCOs have the potential to bind non-enzymatically to free amino groups on proteins and form AGEs. Apart from heat-sterilized, RCOs can originate from uremic circulation and lipids, and become Advanced Lipoxidation End-Products (ALEs). A number of cellular responses are stimulated by AGEs/ALEs and it causes a further increase in oxidative stress. AGEs/ALEs are known to trigger monocyte chemotaxis and apoptosis, secretion of inflammatory cytokines from macrophages, the proliferation of smooth muscle cells and platelet aggregation. AGEs also induce the VEGF and TGF- β1 synthesis from

mesothelial cells, leading to membrane alterations [33]. Accumulation of AGEs has been found in the mesothelium, submesothelial stroma and vascular wall of PD patients. AGEs correlated with the progression of interstitial fibrosis and vascular sclerosis [34]. Furthermore, the degree of AGE accumulation was also correlated with time spent on PD and associated with an increase in peritoneal permeability [35].

Lactate and acidic

Lactate is used as a buffer in PD solutions. Because of the acidic in PD solutions, lactate will alter the level of intracellular calcium and increase acidic in peritoneal cells, leading to mesothelial cells injury. Lactate also increases the production of TGF- β1 and Monocyte Chemoattractant Protein-1 (MCP-1) [36]. Moreover, lactate can reduce the actions of neutrophils and the synthesis of cytokines from leukocytes [37]. Nevertheless, no study confirms the effect of acidic in the fibrosis process.

2.3 Monitoring of the peritoneal membrane status

2.3.1 Monitoring of peritoneal functional changes

The Peritoneal Equilibrium Test (PET)

Peritoneal membrane transport can be measured by various parameters, such as Peritoneal Equilibrium Test (PET), Peritoneal Dialysis Capacity test (PDC), Standard Peritoneal Permeability Analysis (SPA), etc. Among these tests, the most widely used method to evaluate peritoneal transport in PD patients is PET. This is probably because of the simplicity of the test and a highly well controlled in-center procedure which is accurate and reliable over repeated periods of observation. It can be used to adjust the dialysis prescriptions and monitor the changes of peritoneal membrane in PD patients [38, 39].

The principle of this test, which originally described by Twardowski et al [40], is to evaluate low molecular weight solute transfer, using the dialysate over plasma (D/P) ratio of creatinine and the ratio of dialysate glucose concentration. After the dwell of 8-12 hours, the PET is performed during a 4-hour dwell using 2 liters of glucose 2.27% or 2.5% dialysis solution. Dialysate is sampled from the drained effluent before the test, from the test bag at 0, 120 and 240 minutes after drainage, and from the following bag before inflow and immediately after inflow. The serum is sampled at 120 minutes after drainage.

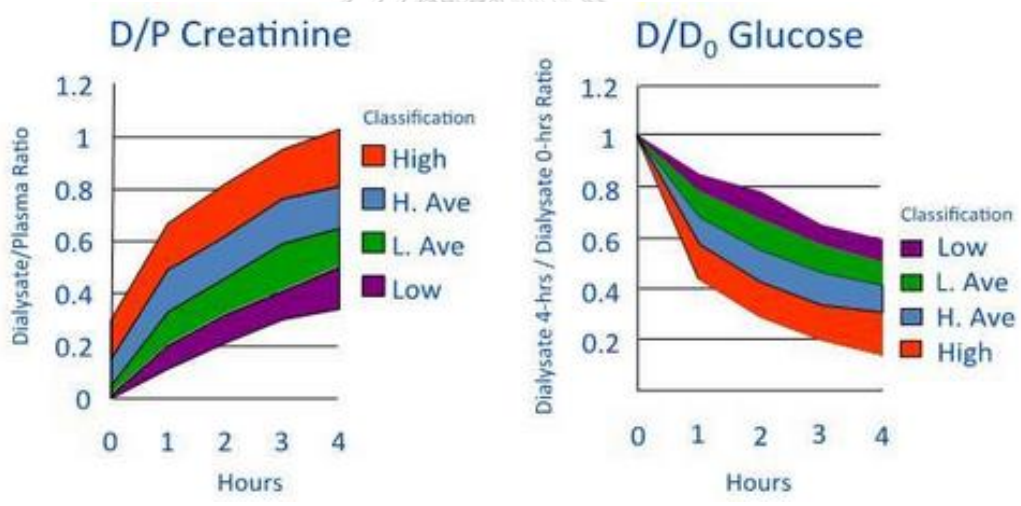
Peritoneal solute transport is calculated by the D/P ratio of creatinine and the dialysate at 0, 120 and 240 minutes /initial dialysate ratio of glucose (D/D_0 glucose). According to the values of solute transport, patients are categorized into 4 groups of low, low-average, high-average and high transporters (Table 1, Figure 2). A high transporter is defined as a patient with either a D/P_{Cr} exceeding the mean +1 SD, or a D/D_0 of less than the mean D/D_0 -1 SD. High average transporters have a D/P_{Cr} between the mean and mean +1 SD, or a D/D_0 between the mean and mean -1SD. Analogously, the other 2 groups are defined [24, 38].

Net ultrafiltration is calculated as the difference between the drained and the instilled volume. Patients are considered to have ultrafiltration failure when net ultrafiltration is less than 100 ml. after 4-hour dwell using glucose 2.27% or 2.5% dialysis solution.

Table 1 Classification of peritoneal dialysis patients by PET results [41]

Transport classification	D/P creatinine	D/D_0 glucose	Net ultrafiltration (mL)
High	0.82-1.03	0.12-0.26	(-470)-35
High average	0.66-0.81	0.27-0.37	35-320
Mean	0.65	0.38	320
Low average	0.50-0.64	0.39-0.48	320-600
Low	0.34-0.49	0.49-0.61	600-1,276

Figure 2 Twardowski Curves: Transport Status Based on the PET [42]



In 2006, the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) clinical practice guidelines for PD adequacy suggested that baseline peritoneal membrane transport characteristics should be performed after initiating PD therapy and it would be best to wait 4 - 8 weeks after starting dialysis because earlier testing may not accurately reflect the transport status of patients. Peritoneal membrane transport should be repeated every 6 -12 months or when patients have clinically indicated such as volume overload, uremia, etc. If patients are

experiencing a peritonitis episode, it should be obtained when the patients are clinically stable and at least 1 month after the resolution of an episode of peritonitis [43].

2.3.2 Biomarkers for monitoring peritoneal membrane changes

Nowadays, monitoring the peritoneal membrane status in PD patients by using the peritoneal membrane transport is commonly used in clinical practice, but there are some disadvantages to this parameter. It is assumed that the peritoneal capillary represents the key barrier to peritoneal transport, therefore, the changes in solute transport reflect the number and possibly ultrastructural changes of these vessels [44]. Thus, peritoneal membrane transport is not a good predictor for membrane deterioration because it does not reflect all pathology of the peritoneal membrane such as the loss of mesothelial cells and EMT process, which are an early event that may initiate other abnormalities. Therefore, measuring the peritoneal effluent biomarkers should be paid more attention because it can represent various pathologic conditions of peritoneal tissues and more early detection of membrane dysfunction.

Cancer Antigen 125 (CA125)

Dialysate CA125 is the most extensively studied biomarker in PD. CA125 is a glycoprotein with a high molecular weight (exceeding 200,000 daltons in gel filtration experiments) [45]. It is produced by mesothelial cells and can be found in peritoneal dialysate effluent in peritoneal dialysis patients. Studies have shown a positive correlation between the concentration of CA125 and the numbers of mesothelial cells in both peritoneal effluent and cultured human mesothelial cells [46, 47]. Thus, CA125 can be used as a biomarker of peritoneal mesothelial cell mass and measurement of CA125 concentration in peritoneal dialysate effluent can be used to monitor the decrease in peritoneal mesothelial cells in PD patients.

Data have shown that the dialysate CA125 concentration increases with a longer dwell time [48-51], possibly due to the linear appearance of mesothelial cells in dialysate or continuous mesothelial turnover in situ; both the growth and death of mesothelial cells can increase CA125 release [51].

The peritoneal membrane structure can change over time when exposed to long-term PD, and peritoneal biopsy data showed a loss of mesothelial integrity after 5 years on PD [21]. This finding is in accordance with the results of subsequent studies that showed that the longer the duration of PD, the lower the CA125 concentration [49, 50, 52].

Dialysate CA125 can be used to determine the biocompatibility of PD solutions since it can represent the effect of PD solutions on mesothelial cell mass. Many studies have investigated the relationship between the biocompatible dialysis solution and dialysate CA125. All studies had the same results, which was that the biocompatible dialysis solution could increase dialysate CA125 [53-56]. Dialysate CA125 concentration has also been studied to use for early detection and evaluation of Encapsulating Peritoneal Sclerosis (EPS). It was also reported that the CA125 appearance rate lower than 33 U/min had a sensitivity of 70% and a specificity of 66% [57].

Interleukin-6 (IL-6) [44]

IL-6 is a pleiotropic cytokine, generated by different cell types such as activated monocyte/macrophages, T cells, mesothelial cells, fibroblasts, and vascular endothelial cells. IL-6 has a molecular weight of 26 kDa and is locally synthesized in the peritoneal cavity during PD, as indicated by the fact that IL-6 concentrations are higher in dialysate than in serum. Dialysate IL-6 concentrations were shown to increase linearly during a peritoneal function test.

Infectious peritonitis causes a dramatic increase in the local production of this cytokine. Correlations between dialysate IL-6 level and peritoneal solute transport have been described in some PD patients without infection, but not in all patients. Variable results have been reported in longitudinal studies with regard to the relationship between dialysate IL-6 concentration and peritoneal solute transport. The inconsistent results might be explained by the presence or absence of low-grade peritoneal inflammation, the incidence and prevalence of which is likely to differ between populations.

Growth factors for angiogenesis [44]

Both VEGF and Connective Tissue Growth Factor (CTGF) are involved in angiogenesis. VEGF is a glycoprotein that is mainly secreted in a soluble form. It increases vascular permeability, as shown by the high concentrations that are present in the ocular fluid of patients with proliferative diabetic retinopathy. The VEGF concentrations in peritoneal effluent are mostly come from local VEGF production or release and are apparently related to peritoneal transport by diffusion of low molecular weight solutes. The diffusion of low molecular weight solutes is dependent on the number of perfused peritoneal microvessels, and thereby on the effective peritoneal vascular surface area. No relationship between local production of VEGF and the duration of PD was found in one cross-sectional study. However, an increase in VEGF during longitudinal follow-up was shown in a study with a small sample of patients, which is according to the progression of neoangiogenesis. The discrepancy results on the effect of the time on PD on VEGF concentration between the cross-sectional observation and the longitudinal study is because of high inter-individual variability.

CTGF is a cysteine-rich peptide with angiogenic properties. There was data showed that PD patients with ultrafiltration failure had higher expression of CTGF mRNA than nondialysed patients with chronic renal insufficiency. A few studies have described

that the local production or release of CTGF possibly determines peritoneal dialysate CTGF concentration. Same as the results of VEGF, there were relationships between dialysate CTGF concentration and the rate of low molecular weight solutes transport, but no relationship was found between dialysate CTGF and the duration of PD.

2.4 Strategies for preserving peritoneal membrane

Attempts have been made to prevent and inhibit peritoneal membrane remodeling during PD, however, there has been no strategies to confirm the efficacy in peritoneal membrane preservation. The strategies that have been studied are as follows.

2.4.1 Use of more biocompatible PD solutions

Due to effects from conventional PD solutions on the peritoneal membrane, biocompatible PD solutions have been developed to reduce membrane deterioration. Biocompatible PD solutions have a more physiologic pH, which contain bicarbonate-lactate buffers and fewer GDPs by using non-glucose osmotic agents such as amino acid and icodextrin.

Neutral-pH, low GDPs solution

This solution is a glucose-based solution uses either bicarbonate buffer, combination of bicarbonate-lactate buffer or lactate buffer with a multicompartment bag system to separate out the buffer from the glucose compartment, so it produces a more physiologically compatible pH of approximately 7.0 and a low concentration of GDPs. However, glucose is still used as an osmotic agent and it has high osmolarity. It was reported that a neutral-pH, low GDPs solution was associated with a significant improvement in the dialysate biomarkers of peritoneal membrane integrity and peritoneal ultrafiltration (UF), decreased circulating AGEs concentrations and signs of

EMT in mesothelial cells from PD effluents [55, 56, 58, 59]. Recent studies have showed that this solution could exert differential effects on peritoneal small solute transport rate and UF overtime [60], and probably also delay the onset of anuria compared with a conventional solution, but do not affect technique failure and patient survival [61]. In a systemic review in 2014, they found that a neutral pH, low GDPs PD solution resulted in the better preservation of residual renal function (RRF) with a more than 12 month follow-up including urine output up to three years of therapy duration. There is no significant effect on peritonitis, technique failure or adverse events [62]. Consistent with another systemic review, only a group of long-term studies (more than 12 months) showing improvement of RRF value when compared with the conventional PD solution [63].

Amino acid-based solution

The amino acid-based solution has been developed in order to increase the protein level and nutritional status of PD patients. Glucose is replaced by amino acids as an osmotic agents, thus, the levels of glucose and GDPs are reduced. A previous study found that it did not cause any toxic effects or worsen the peritoneal membrane function [64]. Moreover, another study showed a better preservation of mesothelial cell mass [65]. However, there are some weakness for this solution because of its adverse effects [66].

Icodextrin-based solution

Icodextrin is a polymer of glucose synthesized by the hydrolysis of cornstarch. Icodextrin-based PD fluid contains relatively low levels of GDPs and is approximately iso-osmolar to serum. When administered intraperitoneally, icodextrin acts as a colloid osmotic agent which leads to net fluid movement from blood to the dialysate. As an osmotic agent, it is as effective as 3.86% glucose solution [67, 68]. Icodextrin is relatively slowly absorbed from the peritoneal cavity. Consequently, the absorption of the osmotic agent is much slower than for glucose, resulting in a longer duration of the

osmotic gradient [69]. Mesothelial cells taken from the PD effluent showed greater proliferation than the glucose-based solution [70]. When compared to glucose-based PD solutions, icodextrin produced increased, maintained UF, improving fluid removal and status, increasing small solute clearance and sodium removal in PD patients [71-73]. In a longitudinal clinical trial, icodextrin had a beneficial effect on technique survival, but there were no obvious benefits or disadvantages in residual renal and peritoneal functions [74]. In the case of using combined icodextrin and biocompatible glucose-containing PD solutions, one icodextrin-containing solution for the long dwell and two exchanges of glucose-containing solutions a day was more biocompatible in terms of glucose exposure and mesothelial cell homeostasis preservation compared to that using four exchanges of glucose-containing solutions [75]. Recently, a systematic review showed that icodextrin solutions were 70% less likely to experience uncontrolled episodes of fluid overload, improved peritoneal UF and had a comparable incidence of adverse events, but no effects of icodextrin on technique or patient survival were observed [62].

2.4.2 Use of Renin-Angiotensin-Aldosterone System (RAAS) blockades

It is believed that local RAAS has a potential role in peritoneal membrane remodeling. Angiotensin II causes peritoneal structural and functional changes by producing peritoneal fibrosis through TGF-β and inducing neoangiogenesis through VEGF. Several studies have demonstrated that factors of membrane deterioration, such as bioincompatible PD solution, peritonitis, and uremia, can cause peritoneal mesothelial cell injury leading to local RAAS activation, especially angiotensin II and aldosterone. Therefore, TGF-β is consequently released by macrophage and fibroblast cells, and eventually result in fibrosis [76]. Several in vitro and in vivo studies have verified the efficacy of angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) in preserving the peritoneal membrane from a bioincompatible PD solution. It was reported that losartan could reduce the up-

regulation of TGF-β from human peritoneal mesothelial cells after stimulated by the high concentration of glucose solution [77]. Giving intravenous valsartan to peritoneal fibrosis-induced rats significantly decreased the expression of membrane damage biomarkers [78]. Moreover, the administration of ACEI and ARB in rats by oral or intraperitoneal route can prevent peritoneal membrane both functional and structural alterations by inhibiting TGF-β and VEGF production [79, 80]. Two recent studies in rats found that intraperitoneal and intravenous renin inhibitor (Aliskiren) improved damage and fibrosis markers, and prevented functional modifications in peritoneal transport [81, 82]. Mineralocorticoid receptor blockade (spironolactone) is also able to ameliorate the progression of peritoneal fibrosis and improve peritoneal membrane function in the peritoneal scraping rat model [83].

In clinical studies, the results are still inconclusive. A retrospective study in PD patients who received ACEI or ARB prevented the increase in peritoneal membrane transport but showed no effect on the UF when compared to the control [84]. Another retrospective study reported that the control group had a decrease in UF and an increase in dialysate TGF-β1, VEGF and fibronectin, while this was not changed in the ACE/ARB group [85]. Contrary to the result in a cross-sectional study, the use of ACE/ARB did not alter dialysate VEGF, TGF-β, IL-6 or peritoneal membrane characteristics test [86]. In a prospective cohort study, ACEI/ARB prevented the increase in small solute transport in long-term PD, and probably have had a positive effect on technique survival, but not on patient survival [87]. The effects of spironolactone on peritoneal function and RRF in PD patients were also studied. A six-month treatment with spironolactone slowed the loss of peritoneal function, suppressed the expected elevation in serum profibrotic markers and increased marker of mesothelial cell mass, but unable to show a positive effect on RRF [88]. However, a two-year treatment study showed no significant difference in RRF and peritoneal transport between spironolactone and control group [89].

2.4.3 Use of glycosaminoglycans supplementation

Glycosaminoglycans are long unbranched polysaccharide chains containing a repeating disaccharide unit. They are large complexes of negatively-charged molecules located primarily on the surface of cells or in the extracellular matrix. The specific GAGs that have physiological significance include hyaluronan, dermatan sulfate, chondroitin sulfate, heparin and heparan sulfate, and keratan sulfate [90]. Glycosaminoglycans are synthesized and secreted by cultured mesothelial cells and found in PD effluent after dialysis exchange. The role of GAGs in maintaining the integrity of the mesothelial monolayer may be due to their physical properties that provide a hydrated and low friction surface, allowing internal organs to move relative to one another, and avoiding the formation of adhesion [91].

Hyaluronan, which is a major component of the extracellular matrix, is produced by mesothelial cells. It has a role in tissue integrity and the maintenance of epithelial cell phenotype, including the anti-angiogenic property, provides structural support to the peritoneal membrane [92]. It was demonstrated that adding high molecular-weight hyaluronan to PD solution exerted anti-inflammatory and anti-fibrotic actions on the in vitro cultured mesothelial cells and accelerated their growth rate [93].

Heparin is a member of the GAGs family that has an anticoagulation effect. In addition to this effect, it has been reported to decrease peritoneal membrane dysfunction in PD patients. There are data that have shown that heparin could increase UF in an animal model [94]. In rats, exposure to PD fluid leaded to the activation of the complement and coagulation. In the case of the intraperitoneal injection of low molecular weight heparin (LMWH), it inhibited complement activation and thrombin formation. Angiogenesis was also inhibited through the inhibition of VEGF and growth factors, resulting in the reduction of inflammation and fibrosis and improvement of UF [95]. In a clinical study, patients were randomized to receive either placebo or tinzaparin intraperitoneally. Peritoneal membrane solute transport was reduced in patients who

received tinzaparin, along with an increase in UF volume and a decrease in dialysate IL-6 concentration [96].

2.4.4 Prevention and management of peritonitis [97]

Because of severe or prolonged peritonitis leads to structural and functional alterations, therefore, prevention and management of peritonitis is an important issue to reduce peritoneal membrane dysfunction.

Numerous prevention strategies aim to reduce the incidence of exit-site and catheter- tunnel infections. International Society for Peritoneal Dialysis (ISPD) guideline for peritonitis has recommended that systemic prophylactic antibiotics should be administered immediately prior to catheter insertion. Peritoneal dialysis patients and their caregivers should approach training programs conducted by nursing staff with the appropriate qualifications and experience. They also recommend daily topical antibiotic application (mupirocin or gentamicin) cream or ointment to the catheter exit site and instant treatment of exit-site or catheter tunnel infection to reduce subsequent peritonitis risk.

For the management of peritonitis, ISPD recommend identifying causative organism by using the bacterial culture of peritoneal dialysate effluent. Empirical antibiotic therapy should be initiated as soon as possible after appropriate microbiological specimens have been obtained. Empirical antibiotic regimens should be center specific and cover both gram-positive and gram-negative organisms. Gram-positive organisms should be covered by vancomycin or a first-generation cephalosporin and gram-negative organisms should be covered by a third-generation cephalosporin or an aminoglycoside. Antibiotic therapy should be changed to narrow-spectrum agents after culture results and sensitivities are known. Peritoneal dialysis catheter could be removed promptly in refractory peritonitis episodes, defined as a failure of the PD effluent to clear up after 5 days of appropriate antibiotics.

2.5 Roles of sulodexide in peritoneal membrane preservation

Sulodexide is a mixture of glycosaminoglycan consisting of fast moving heparin 80% and dermatan sulfate 20%. Fast moving heparin is characterized by a low-medium molecular weight (700D), lower sulfation degree, lower anticoagulant activity than the slow-moving heparin fraction and unfractionated heparin. Dermatan sulfate is a polydisperse polysaccharide is responsible for its anticoagulant, specifically antithrombin activity, and for its antithrombotic activity [11].

Glycosaminoglycans are long unbranched polysaccharide chains containing a repeating disaccharide unit. They are large complexes of negatively-charged molecules located primarily on the surface of cells or in the extracellular matrix. The specific GAGs that have physiological significance include hyaluronan, dermatan sulfate, chondroitin sulfate, heparin and heparan sulfate, and keratan sulfate [90]. Glycosaminoglycans are synthesized and secreted by cultured mesothelial cells and found in PD effluent after dialysis exchange. The role of GAGs in maintaining the integrity of the mesothelial monolayer may be due to their physical properties that provide a hydrated and low friction surface, allowing internal organs to move relative to one another, and avoiding the formation of adhesion [91].

2.5.1 Pharmacokinetics of sulodexide

Sulodexide has a high bioavailability after intramuscular, intravenous or oral administration. Oral sulodexide is absorbed within 1-2 hours. The bioavailability of the oral route is in the range of 40% - 60%. The peak plasma concentration of sulodexide is 0.2-1.0 mg/L at 1-10 hours after oral administration [98]. It is excreted through the bile 23% and through the kidney 55%. The elimination half-life of sulodexide is 11.7$\pm$2.0 hours after 50 mg intravenous administration, 18.7$\pm$4.1 hours after 50 mg oral administration and 25.8+1.9 hours after 100 mg oral administration [11, 98].

2.5.2 Adverse effects of sulodexide

Oral administration of sulodexide is extremely well tolerated in humans and in animals, and the adverse reactions described after oral administration are related mainly to transient gastrointestinal intolerance such as nausea, dyspepsia and minor bowel symptoms [98].

2.5.3 Mechanisms of sulodexide in peritoneal membrane preservation

Sulodexide has been used as an antithrombotic drug and to reduce proteinuria in patients with diabetic nephropathy. Apart from these indications, it has been reported to decrease peritoneal membrane dysfunction in PD patients. The mechanism of sulodexide in maintaining the peritoneal membrane structure and function is still not completely clear, but it could be the same mechanism as nephroprotective action in diabetic nephropathy because of the same pathology. The mechanisms are reducing TGF-β1 and VEGF synthesis, matrix synthesis, inflammation, cellular proliferation and EMT [20]. The anti-inflammatory effect of sulodexide is attributed to its antithrombin action. The fast moving heparin and dermatan sulfate fractions of sulodexide accelerate the inhibition of thrombin by their simultaneous interactions with antithrombin III and heparin cofactor II, respectively [12]. Antithrombin III induces prostacyclin generation in endothelial cells by interacting with heparan sulfate of endothelial cells and inhibits cytokine and tissue factor production in endothelial cells and monocytes. Similar mechanisms may be involved in cellular actions of antithrombin III causing desensitization of chemoattractant receptors of leukocytes by activating the heparan sulfate proteoglycan [99].

2.5.4 Studies of sulodexide in peritoneal membrane preservation

Heparin sodium and low molecular weight heparins (LMWH) are in the same glycosaminoglycan family as sulodexide. There is data showed that heparin could

increase ultrafiltration in an animal model [94]. In rats, exposure to PD fluid induced activation of the complement and coagulation by detecting the formation of thrombin-antithrombin complex. In the case of LMWH IP injection, complement activation and thrombin formation were inhibited. Angiogenesis was also inhibited through the inhibition of VEGF and growth factors, resulting in reduced inflammation and fibrosis, and increased the intraperitoneal fluid volume, indicating improved ultrafiltration [95]. In a randomized cross-over study with 2 treatment periods of 3 months, 21 PD patients were randomized to receive either placebo or tinzaparin intraperitoneally. Patients in the tinzaparin period had a reduction in D/P of creatinine, urea and albumin along with an increase in ultrafiltration volume and a decrease in dialysate IL-6 [96, 100].

There are 2 small uncontrolled clinical studies of sulodexide, the first study [16], sulodexide was administered intraperitoneally for 1 month in 16 long-term PD patients. It was reported the decrease in peritoneal protein loss and increase in D/P of urea and creatinine. The second study was done in 6 PD long-term patients. Patients were received oral treatment of 25-125 mg of sulodexide for 5 months by titrating doses every month. Increasing of D/P urea and creatinine were found and dose-dependent reduction of IL-6, IL-8, and IL-1β in the dialysis fluid was induced by sulodexide [17]. Both studies reported that no patients had coagulation disorders, hemorrhages or side effects throughout the studies. The inconsistency with LMWH treatment can be explained by inadequate study designs such as short intervention periods and lack of randomized placebo groups. Moreover, methods for the determination of creatinine in plasma and dialysate were different.

Animal studies have shown that functional and morphological alterations induced by plasticizers were prevented by the SC injection of sulodexide; indeed, it reduced the damage to the peritoneal structure, and maintained an almost normal peritoneal efficiency, as shown by normal ultrafiltration, transport of urea, and albumin clearance [13]. A study in rats with acute peritonitis, IM sulodexide was given, the

dialysate cell count and dialysate elastase activity were lower compared to the peritonitis group. In rats treated group, the increase of plasma TNF-α was reduced. Pretreatment with sulodexide reduced the transperitoneal loss of total protein and albumin during peritonitis [14]. A recent study demonstrated that oral sulodexide administration diminishes neo-vascularization, submesothelial thickening and EMT induced by exposure to PD fluid in a rat model. Creatinine and glucose transport were better preserved in the sulodexide group versus control [15].

CHAPTER III

RESEARCH METHODOLOGY

3.1 Study samples

This study was conducted during 2014 at the Department of Medicine, Phramongkutklao Hospital, and Banphaeo Hospital (Prommitr branch), Bangkok, Thailand. It was approved by the institutional review boards and ethics review committees of the Royal Thai Army Medical Department, Phramongkutklao Hospital and College of Medicine, Bangkok, Thailand (No. Q022h/56) (Appendix A). Patients were included in this study with the following criteria:

Inclusion criteria

1. End-stage renal disease patients undergoing CAPD with conventional PD solution for at least 6 months
2. Male or female patients 20 years and older

Exclusion criteria

1. Previous therapy with sulodexide or heparin in the previous 1 month
2. Patients with infectious peritonitis or had more than 1 peritonitis episode or had peritonitis episode in the 3 months before the study
3. Patients with high peritoneal solute transport (D/P creatinine exceeding 0.81 or D/D_0 glucose less than 0.27)
4. Patients with coagulopathy or on anticoagulant drug therapy
5. Pregnant or planning to become pregnant or lactating females
6. Patients with hepatic disease or liver enzymes values exceeding 5-fold above the normal value

7. Patients with cancer diseases or immunodeficiency
8. Bedridden Patients
9. Patients with severe or exacerbation of cardiovascular disease
10. Patients with malnutrition
11. Refusal or unable to provide informed consent

3.2 Sample size calculation

This study investigates 2 independent samples. The primary outcome is to compare dialysate CA125, IL-6 and VEGF levels between 2 groups. The sample size calculating formula for mean difference as followed;

$$n/group = \frac{2(Z_{\alpha/2} + Z\beta)^2 \sigma^2}{d^2}$$

CA 125

Define, n/group = sample size in each group, α = 0.05, β = 0.20, $Z_{\alpha/2} = Z_{0.05/2}$ = 1.96(2-tailed), $Z\beta = Z_{0.20}$ = 0.84, d = the difference in dialysate CA125 level between treatment and control group was 12 U/ml, data based on previous study by Khunprakant R. [101] σ^2 = variability of endpoint derived from the following calculation.

Pooled variance (Sp^2), using data from previous studies [101].

$$Sp^2 = \frac{S_1^2 + S_2^2}{2}$$

$$Sp^2 = \frac{(10.6)^2 + (19.3)^2}{2} = 242.42$$

$$n/group = \frac{2(1.96 + 0.84)^2\ 242.42}{12^2}$$

$$= 26.40 \sim 27 \text{ patients/group}$$

IL-6

Define, n/group = sample size in each group, α = 0.05, β = 0.20, $Z_{\alpha/2} = Z_{0.05/2}$ = 1.96(2-tailed), $Z\beta = Z_{0.20}$ = 0.84, d = the difference in dialysate IL-6 level between treatment and control group was 18.4 pg/ml, data based on previous study by Fusshoeller A. [102] σ^2 = variability of endpoint derived from the following calculation.

Pooled variance (Sp^2), using data from previous studies [102].

$$Sp^2 = \frac{S_1^2 + S_2^2}{2}$$

$$Sp^2 = \frac{(21.3)^2 + (15.0)^2}{2} = 339.35$$

$$n/group = \frac{2(1.96 + 0.84)^2\ 339.35}{18.4^2}$$

$$= 15.72 \sim 16 \text{ patients/group}$$

VEGF

Define, n/group = sample size in each group, α = 0.05, β = 0.20, $Z_{\alpha/2} = Z_{0.05/2}$ = 1.96(2-tailed), $Z\beta = Z_{0.20}$ = 0.84, d = the difference in dialysate VEGF level between treatment and control group was 0.17 µg/overnight bag, data based on previous study by le Poole CY. [53] σ^2 = variability of endpoint derived from the following calculation.

Pooled variance (Sp^2), using data from previous study by le Poole CY. [53]

$$Sp^2 = \frac{S_1^2 + S_2^2}{2}$$

$$Sp^2 = \frac{(0.21)^2 + (0.09)^2}{2} = 0.0261$$

$$n/group = \frac{2(1.96 + 0.84)^2\ 0.0261}{0.17^2}$$

$$= 14.16 \sim 15 \text{ patients/group}$$

Use the sample size from CA125 calculation which is the maximum value, calculate for the drop-out rate as followed

Drop-out rate (R) = 15 % ;

$$n/group^* = \frac{n/group}{(1-0.15)} = \frac{27}{0.85}$$

$$n/group^* = 31.8 \sim 32 \text{ patients/group}$$

Thus, patients needed for this study was at least 64 patients, divided into 2 groups (sulodexide or placebo group).

3.3 Data collection

3.3.1 Patients screening

1. Enrolled the participants who meet all eligibility criteria. An information sheet and informed consent were obtained before collecting the patient's data. The consent form included data about study details, objectives of the study, study process, instructions, expected benefit, and probable risk. Participants were informed that all data were collected for scientific research only and kept confidential.

2. Collected patient's demographics and baseline characteristics data in the registration record form (Appendix B). Data collection was as follows.

- Demographics/baseline characteristic data by interviewing and medical record
- Peritoneal dialysis information by interviewing and medical record
- Physical examination by a physician
- Medication history by interviewing and medical record

3.3.2 Treatment

The study drug

1. Randomly assigned the patients to receive either sulodexide (Vessel®, Alfa Wassermann, Italy) or placebo by using a permuted block of 4. Patients did not know which treatment patients received (blinding). The intervention of each group was as follows.

Sulodexide group: Patients were assigned to take the recommended FDA approved dose of sulodexide 50 mg 2 times daily before meal. Soft-gelatin capsule containing 25 mg of sulodexide was contained in capsule size 0, providing in light brown bottles. Patients took 2 capsules per time, 2 times per day, orally with a glass of water 30 minutes before breakfast and dinner for 90 days.

Placebo group: Patients were assigned to take placebo, which was similar color and appearance to study drug produced by Z Natural Pharmaceutical Co.Ltd, Bangkok, Thailand. Patients took 2 capsules per time, 2 times per day, orally with a glass of water 30 minutes before breakfast and dinner for 90 days.

2. The investigator dispensed the study drug and instruct the patient to take the drug as mentioned above. The investigator also instructed the patient to take the drug exactly as prescribed. The patient was instructed to contact the investigator if he/she is unable for any reason to take the drug as prescribed. The patient was also instructed how to manage if he/she missing a dose. If the patient forgot to take it before meal, patient could take it 2 hours after that meal and take the next dose as normal.

3. After the patient completed 90 days of treatment, discontinued both sulodexide and placebo.

Background therapy

1. Throughout the study period, patients' dialysis prescription should not be changed. If necessary, it must be under the discretion of the physician and always record the change.

2. Other aspects of patients' care (e.g. hypertension, lipid, diabetes, anemia, and mineral metabolism) followed the routine clinical practice, with emphasis on the targets in clinical practice guidelines.

Prohibit concomitant treatment

The use of following medications may interfere with the evaluation of safety and tolerability. Therefore, the medication excluded throughout the study was the anticoagulant drug, including warfarin, heparin and low molecular weight heparin. If patient took any of these medications during the course of the study, patient would generally not be discontinued from the study drug, except if it was required to maintain the patient's safety.

3.3.3 Visit schedule and assessments

The patient was scheduled to follow-up every 30 days as follows.

Visit 1 (day 0)

1. Collected data by interview and medical record in monitoring record form (Appendix C)

2. The peritoneal fluid sample was collected from an overnight dwell bag (8-10 hours). After the peritoneal fluid had drained completely at the hospital, the fluid sample was measured the levels of dialysate CA125, IL-6, VEGF.

3. Blood sample (10 mL) was collected to examine as follows.

- Serum biochemical parameter : albumin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Serum Creatinine (Scr), Blood Urea Nitrogen (BUN) and serum electrolytes (sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium)

- Hematology : Hemoglobin (Hb), Hematocrit (Hct), Platelets (Plt), activated Partial Thromboplastin Time (aPTT) and Prothrombin Time (PT)

4. Peritoneal membrane function was evaluated by 4-hour PET, using a 2.5% glucose PD solution. Glucose, creatinine, and protein in the peritoneal fluid were determined.

5. Patients received sulodexide or placebo according to their group for 30 days.

Visit 2 (day 30)

1. Collected data by interview and medical record in monitoring record form. Medication adherence and adverse events were assessed by the investigator.

2. Blood sample (10 mL) was collected to examine as follows.

- Serum biochemical parameter : AST, ALT, Scr, BUN and serum electrolytes (sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium)

- Hematology : Hb, Hct, Plt, aPTT and PT

3. Patients received sulodexide or placebo according to their group for 30 days.

Visit 3 (day 60)

1. Collected data by interview and medical record in monitoring record form. Medication adherence and adverse events were assessed by the investigator.

2. Blood sample (10 mL) was collected to examine as follows.

- Serum biochemical parameter : AST, ALT, Scr, BUN and serum electrolytes (sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium)

- Hematology : Hb, Hct, Plt, aPTT and PT

3. Patients received sulodexide or placebo according to their group for 30 days.

Visit 4 (day 90)

1. Collected data by interview and medical record in monitoring record form. Medication adherence and adverse events were assessed by the investigator.

2. The peritoneal fluid sample was collected from an overnight dwell bag (8-10 hours). After the peritoneal fluid had drained completely at the hospital, the fluid sample was measured the levels of dialysate CA125, IL-6, VEGF.

3. Blood sample (10 mL) was collected to examine as follows.

- Serum biochemical parameter : AST, ALT, Scr, BUN and serum electrolytes (sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium)

- Hematology : Hb, Hct, Plt, aPTT and PT

4. Peritoneal membrane function was evaluated by 4-hour PET, using a 2.5% glucose PD solution. Glucose, creatinine, and protein in the peritoneal fluid were determined.

Note : In each visit, patient received record forms about adverse events and peritoneal PD solutions exchange (Appendix D). Patient was asked to record data at home and bring the form back to investigator in the next visit.

Medication adherence monitoring

Adherence was assessed by the investigator at each visit using pill counts and interview. This information was recorded in monitoring record form. Patient adherence should be at least 80% during the study period. The investigator counseled the patient if compliance was below 80%. The percentage of adherence was calculated as follows.

$$\frac{\text{No. of Pills Absent in Time}}{\text{No. of Pills Prescribed for Time}} \times 100 = \%\ \text{Adherence}$$

Telephone monitoring

While patients were at home, they were monitored adverse events and medication adherence by telephone every 2 weeks. Patients were allowed to ask the investigator for more detail of the study, reported any adverse events or problems.

3.4 Outcome measurement

3.4.1 Efficacy measurement

Clinical studies :

- Peritoneal fluid and blood sample: On visit 1 and 4, the patient was determined peritoneal membrane transport by using PET with 4-hour dwell of 2 liters of glucose 2.5% PD solution. Peritoneal fluid was sampled from the drained effluent before the test, from the test bag at 0, 120 and 240 minutes after drainage. The serum was sampled at 120 minutes after drainage. Peritoneal membrane transport was calculated by D/P of creatinine, D/D_0 of glucose. Net ultrafiltration was calculated as the difference between the drained and the instilled volume. Serum albumin, albumin excretion was also determined.

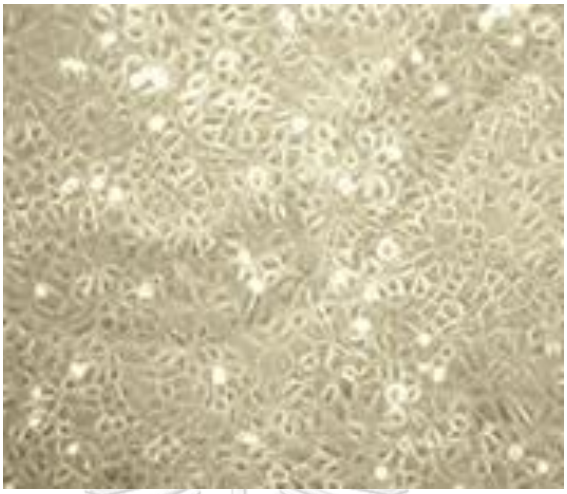
- Overnight dwell bag of PD solution: At visit 1-4, peritoneal fluid drained completely after an overnight dwell to determine effects on biomarkers of membrane remodeling. The drain bag was turned upside-down several times and collected at least 20 ml from the bag. It was centrifuged to remove sediment and frozen in aliquots at -70°C until assay. Dialysate CA125, IL-6 and VEGF concentration, which were selected biomarkers, were measured by Enzyme-Linked Immunosorbent Assay (ELISA) (Appendix E). A polyclonal antibody specific for CA125, IL-6, VEGF were pre-coated onto a microplate. Standards and peritoneal fluid samples were pipetted into the wells and any biomarker present is bound by the immobilized antibody. Biotinylated polyclonal antibody specific for CA125, IL-6, VEGF were added as primary antibody. The streptavidin-HRP conjugate was used as a secondary antibody. Substrate solution was added and color developed. After adding stop solutions, The intensity was measured at 450 nm.

Ex vivo studies :

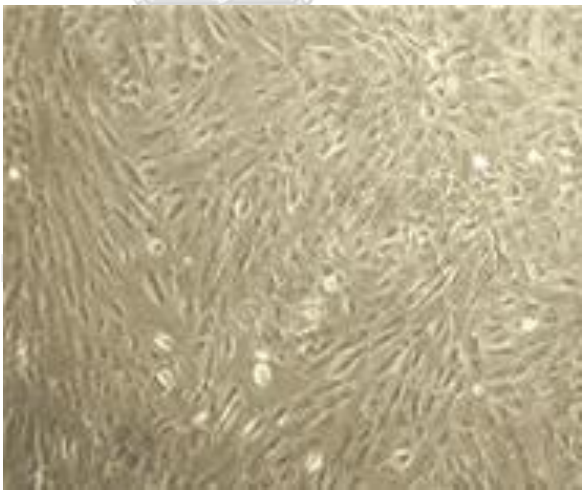
On visit 1 and 4, effluent-derived peritoneal mesothelial cell culture was done by isolating from overnight dwell bag of PD solution. The remaining volume of the drain peritoneal fluid bag, after 20 mL had been drawn for dialysate biomarkers measurements (as mentioned above), was drained into a 50-mL centrifuge tube and cells were concentrated by centrifugation at 500 g for 5 minutes. Cell pellets were seeded onto a 6-well plate and incubated in a humidified 5% CO_2 atmosphere at 37ºC. The culture medium was M199 supplemented with 20% fetal bovine serum, 100 IU/mL penicillin, 100 mcg/mL streptomycin, and replaced every 3 days.

When they nearly reach confluence, the effects on epithelial-to-mesenchymal transition (EMT) of mesothelial cells were determined. EMT is the early event of peritoneal structural change that results in fibrosis and angiogenesis with functional deterioration. The cell scores, which are based on morphologic classification, were done under the light microscope. Cell scores were measured blindly by the pathologists as follows.

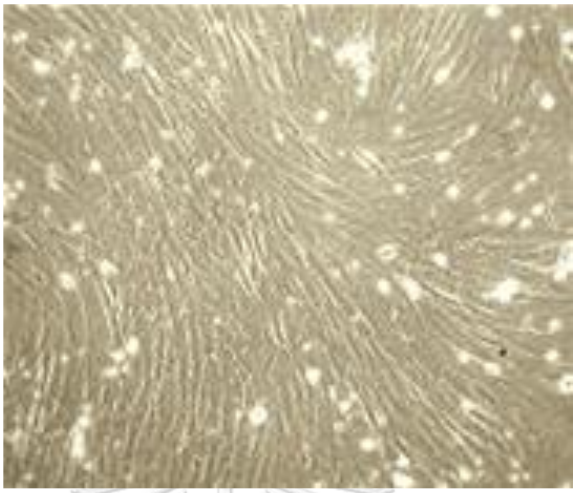
score 1 = cobblestone –shaped (epithelioid phenotype)



score 2 = mixed



score 3 = fibroblast-shaped (fibroblastoid phenotype)



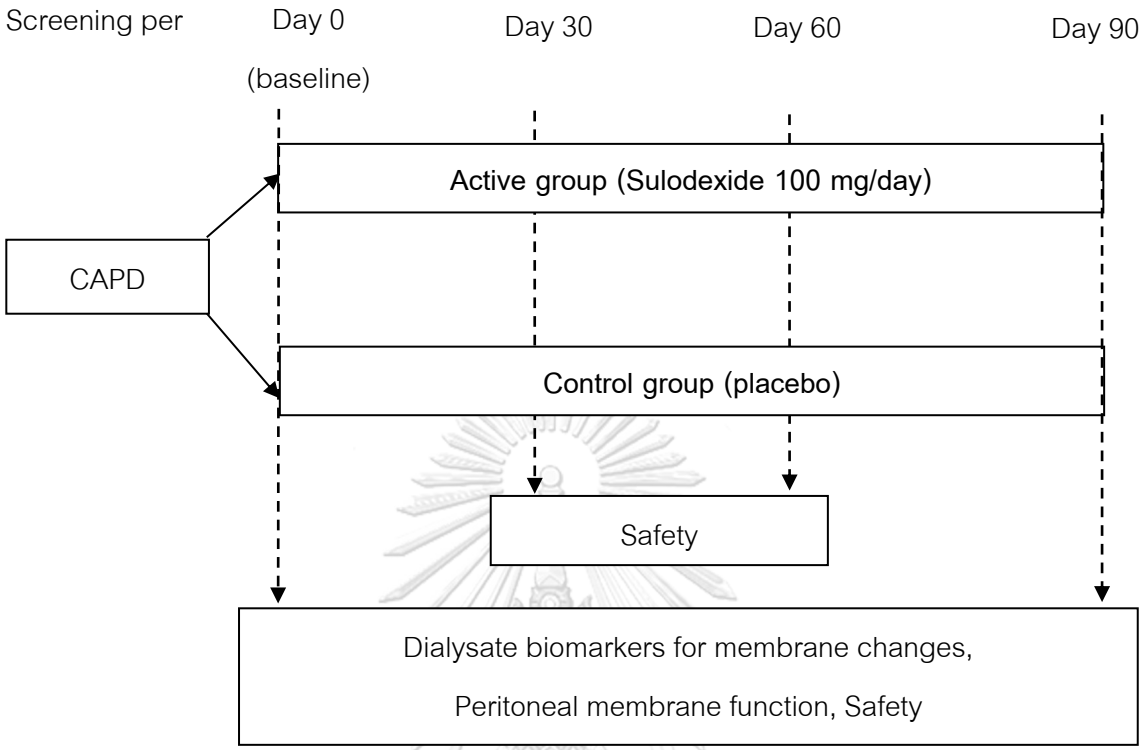
The percentage of epithelioid and fibroblastoid phenotypes and mean cell scores in each study group were calculated.

3.4.2 Safety measurement

- Adverse events were evaluated by Naranjo's algorithm using a self-applied record form and interview.
- Physical examination was performed by the physician on visit 1 and 4
- Laboratory evaluations, which were the change of AST, ALT, Scr, serum electrolytes, Hb, Hct, Plt, aPTT and PT after treated by study drug, were performed on visit 1 and 4.

If an adverse event was detected, it was followed until its resolution. Changes in severity, the suspected relationship to the study drug, the intervention required to treat it or monitor it and outcome assessments were made at each visit.

Figure 3 Flowchart shows the process of the study



3.5 Statistical analysis

The statistical analysis was performed using the SPSS version 17.0 (SPSS. Co., Ltd., Bangkok Thailand) defined significant levels at $\alpha = 0.05$

1. Descriptive statistics showed the frequency, percentage, mean ± SD or median with interquartile ranges depending on a normality test and also tested for homogeneity of the nominal demographic data between groups by Chi-square test or Fisher's exact test.

2. Inferential statistics, which were used to test hypotheses, were shown in the following table.

Table 2 Statistical testing in this study

Hypothesis	Statistical testing
1. CAPD patients treated with sulodexide group had different dialysate CA125, IL-6 and VEGF levels from control group.	Intragroup comparison - Wilcoxon signed - rank test Intergroup comparisons - Mann-Whitney U test
2. Sulodexide- treated group had different D/P creatinine, D/D_0 glucose, net ultrafiltration from control group.	Intragroup comparison - Wilcoxon signed - rank test Intergroup comparisons - Mann-Whitney U test
3. Sulodexide- treated group have no difference in adverse event rates as compared to control group.	Chi-square test

3.6 Ethical Consideration

This study was a randomized placebo-controlled clinical trial. Patients had a chance to receive either sulodexide or placebo. Thus, the investigator realized the risk of receiving the active drug which may have adverse events such as nausea, dyspepsia and minor bowel symptoms. This study needed to draw a blood sample from the patient, so it may cause pain and bruise. Therefore, the investigator described the chance in receiving active drug or placebo, risk of receiving active drug and details of blood and peritoneal effluent sample collection. Moreover, the investigator concerned about the rights of patients to be or not to be participated in the study according to their willingness. All patients were given oral and written information about the study before recruitment and fully described for the objectives and the process of the study by information sheet before deciding to participate in the study. Patients could leave the study anytime which were not impact on the regular treatment they would receive. Data

is kept confidential and presented only the overall results. The study was approved by the Institutional Review Board Royal Thai Army Medical Department before starting the research conduction.

CHAPTER IV

RESULTS

A total of 66 patients, divided into 33 patients for each group, were included in this randomized control trial study. There were 5 patients dropped out of the study (3 patients because of peritonitis and 2 patients because of adverse events tolerance). Overall, 61 (92.42%) patients completed the 3-month study (30 patients in the sulodexide group, 31 patients in the placebo group).

4.1 Participants' demographic data

Baseline characteristic data from patients in each group are shown in Table 3. Most of the patients were female in both groups. Average age of patients was around 50 years old. Hypertension was the most common comorbid disease in both groups. The two groups were similar for all characteristics, including age, duration of PD, comorbid disease, blood pressure, liver function test, previous peritonitis episode, ACEI/ARB treatment which is believed to have a beneficial effect in preserving peritoneal membrane and peritoneal dialysis adequacy (total weekly Kt/v). Overall, an average total weekly Kt/v of patients was in the normal range.

Table 3 Baseline clinical characteristic of study patients

Characteristic data	Sulodexide (n=33)	Placebo (n=33)	p-value
Female (n, %)	19 (57.6)	17 (51.5)	0.75
Age (years)	56.91 ± 8.24	53.94 ± 7.62	0.55
Body weight (kg)	55.91 ± 11.82	59.76 ± 10.65	0.74
Height (cm)	155.0 ± 7.7 [140.0 – 173.0]	157.7 ± 9.2 [144.0 – 178.0]	0.82
Duration of PD (months)	9.8	11.1	0.47
Comorbid diseases (n, %)			0.51
Hypertension	24 (72.7)	28 (84.8)	
Diabetes mellitus	16 (48.5)	14 (42.4)	
Dyslipidemia	17 (51.5)	16 (48.5)	
Coronary artery disease	4 (12.1)	6 (18.2)	
Others	7 (21.2)	10 (30.3)	
Using ACEI/ARB	9	12	0.43
Systolic blood pressure (mmHg)	134.05 ± 19.27	138.26 ± 19.84	0.69
Diastolic blood pressure (mmHg)	83.18 ± 15.63	88.40 ± 15.19	0.14
AST (u/l)	28.9 (10.7)	23.5 (7.4)	0.44
ALT (u/l)	25.2 (15.4)	24.3 (15.7)	0.86
Serum albumin (gm/dl)	3.5 ± 0.4	3.4 ± 0.6	0.92
Total weekly Kt/v	1.9 ± 0.5 [1.3 – 2.5]	1.8 ± 0.5 [1.3 – 2.4]	0.38
Patients with previous peritonitis (n, %)	11 (33.3)	15 (45.4)	0.31

*p-value < 0.05

4.2 Peritoneal membrane changes

4.2.1 Peritoneal transport functions

Peritoneal transport functions were assessed by using a 4-hour peritoneal equilibrium test (Table 4). Results from per-protocol analysis were reported. After the treatment period, there was a significantly lower D/P creatinine in the sulodexide group than in the placebo group (p-value = 0.04). However, no significant difference in D/D0 glucose was observed between the two groups. For 4-hour ultrafiltration volume, there was a significantly higher volume in the sulodexide group when compared to the placebo group (p-value = 0.01). In addition, changes at end point for each of the parameters were also calculated. The significant differences were also found only in D/P creatinine and 4-hour ultrafiltration volume. Increased D/P creatinine from baseline in the placebo group was significantly greater than the change in the sulodexide group (p-value = 0.02). 4-hour ultrafiltration volume decreased from baseline in both groups and this decrease in the placebo group was significantly greater than in the sulodexide group (p-value = 0.02). However, the change from baseline in D/D0 glucose did not significantly differ in both groups.

Furthermore, D/D0 glucose between 2 groups was assessed in subgroup analysis by diabetes mellitus status at baseline (Table 5). No significant difference in D/D0 glucose was found both in diabetes and non-diabetes patients. Likewise, when compared the difference between changes from baseline within both subgroups, it did not reach statistical difference.

Table 4 Peritoneal transport and ultrafiltration characteristics in sulodexide and placebo group

Parameters		Sulodexide (n=30)	Placebo (n=31)	p-value
D/P creatinine	Baseline	0.62 ± 0.09	0.63 ± 0.06	0.62
	After treatment	0.65 ± 0.08	0.70 ± 0.09	0.04*
	Change at end point	0.03 ± 0.04	0.08 ± 0.03	0.02*
D/D0 glucose	Baseline	0.37 ± 0.12	0.38 ± 0.12	0.81
	After treatment	0.41 ± 0.13	0.39 ± 0.12	0.35
	Change at end point	0.03 ± 0.05	0.02 ± 0.07	0.32
4-hour Ultrafiltration (mL)	Baseline	777.4 ± 268.6	799.3 ± 243.6	0.08
	After treatment	657.7 ± 341.0	632 ± 291.9	0.01*
	Change at end point	-110.2 ± 53.4	-158.4 ± 86.1	0.01*

D/P creatinine = dialysate-to-plasma ratio of creatinine, D/D0 glucose = dialysate-to-initial dialysate concentration ratio of glucose, *p-value < 0.05

Table 5 D/D0 glucose characteristics subgroup by diabetes status in sulodexide and placebo group

Parameters		Diabetes patients (n=28)		p-value	Non-diabetes patients (n=33)		p-value
		Sulodexide (n=15)	Placebo (n=13)		Sulodexide (n=15)	Placebo (n=18)	
D/D0 glucose	Baseline	0.39 ± 0.14	0.41 ± 0.18	0.09	0.36 ± 0.16	0.37 ± 0.2	0.71
	After treatment	0.42 ± 0.19	0.40 ± 0.16	0.11	0.39 ± 0.17	0.37 ± 0.15	0.40
	Change at end point	0.02 ± 0.02	0.01± 0.02	0.46	0.04 ± 0.03	0.01± 0.01	0.06

D/D0 glucose = dialysate-to-initial dialysate concentration, *p-value < 0.05

4.2.2 Peritoneal biomarkers

Overnight peritoneal biomarkers changes between the two groups were also analyzed in the per-protocol analysis (Table 6). After the treatment period, patients in sulodexide groups had no significant difference change from baseline in peritoneal CA125 concentration while there was a significantly lower CA125 concentration in the placebo group (p-value = 0.03). However, no significant difference in CA125 concentration was found between the two groups. (Figure 4). For peritoneal IL-6 concentration (Figure 5), a significantly higher level was found within the placebo group after the treatment period (p-value < 0.01) while there was no significant difference change within the sulodexide group. When compared between groups, a significantly higher IL-6 concentration was found in the placebo group than those in the sulodexide group (p-value = 0.03). No significant difference was observed for peritoneal VEGF concentration changes both within and between two groups of patients (Figure 6).

Table 6 Peritoneal effluent biomarkers in sulodexide and placebo group

Biomarkers		Sulodexide (n=30)	Placebo (n=31)	p-value
CA125	Baseline	25.8 ± 12.9	28.7 ± 17.5	0.81
	After treatment	25.1 ± 10.7	24.6 ± 13.3	0.55
IL-6	Baseline	79.2 ± 11.3	82.4 ± 10.1	0.38
	After treatment	80.6 ± 10.8	88.7 ± 11.5	0.03*
VEGF	Baseline	15.9 ± 4.9	14.2 ± 3.6	0.63
	After treatment	15.6 ± 6.2	16.1 ± 4.4	0.82

*p-value < 0.05

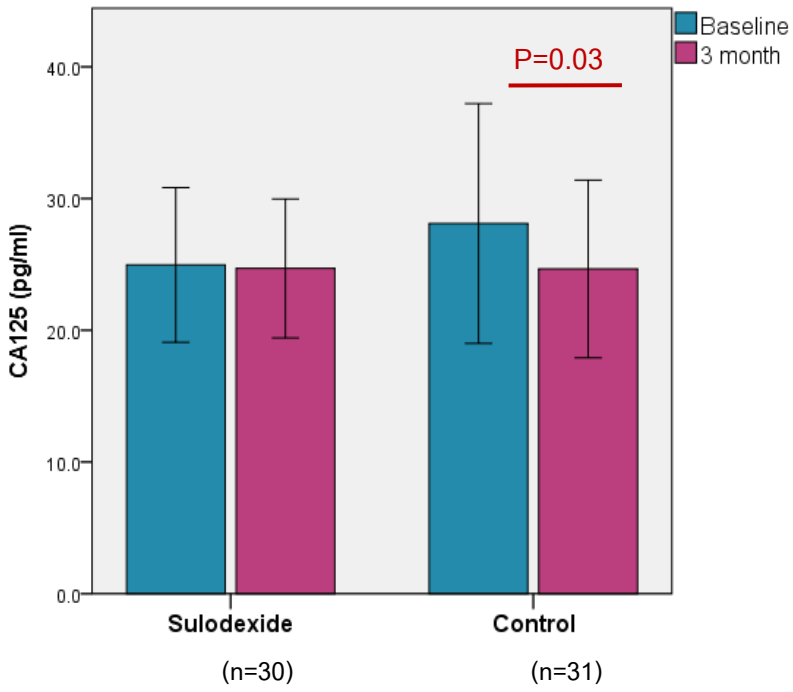


Figure 4 Effect of sulodexide on levels of CA125 in peritoneal effluents

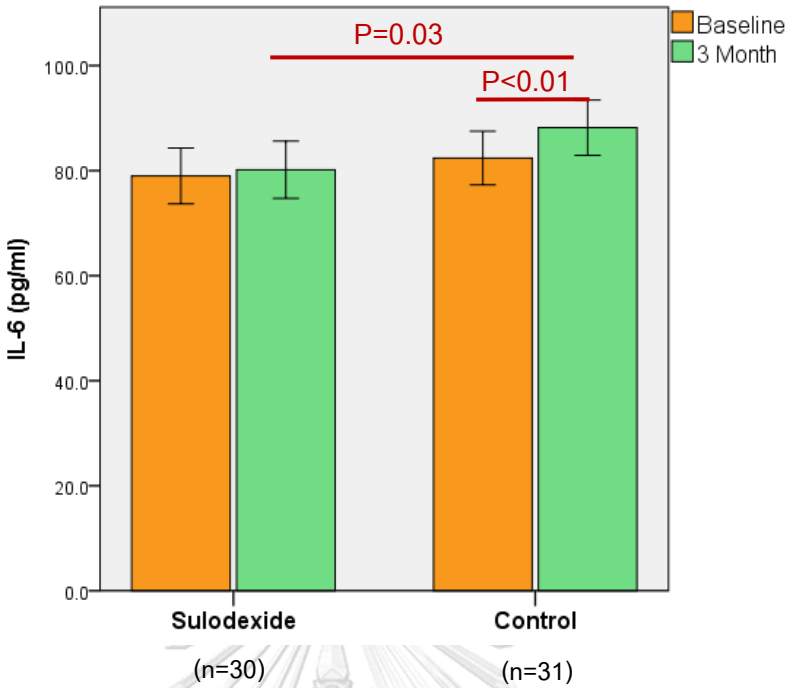


Figure 5 Effect of sulodexide on levels of IL-6 in peritoneal effluents

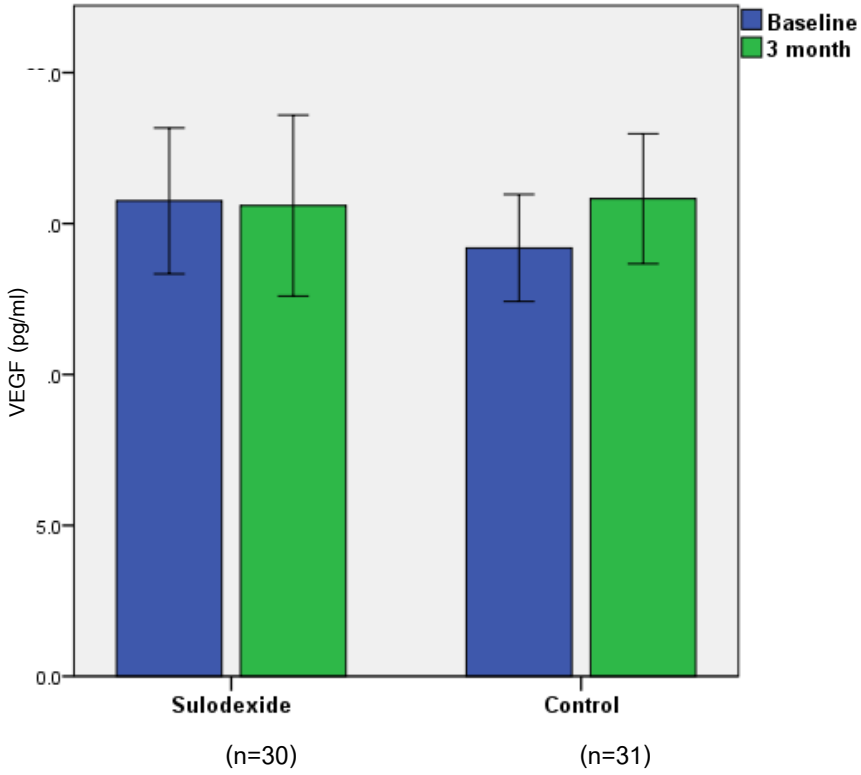
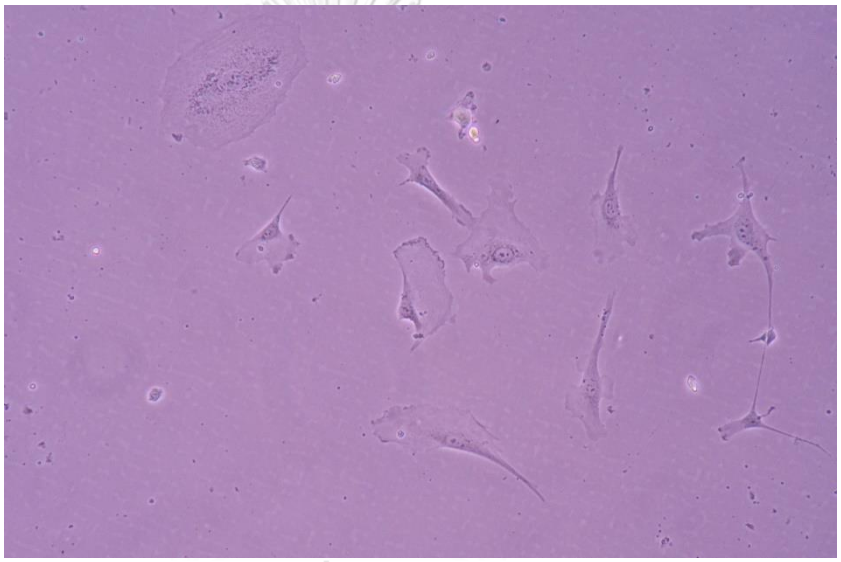


Figure 6 Effect of sulodexide on levels of VEGF in peritoneal effluents

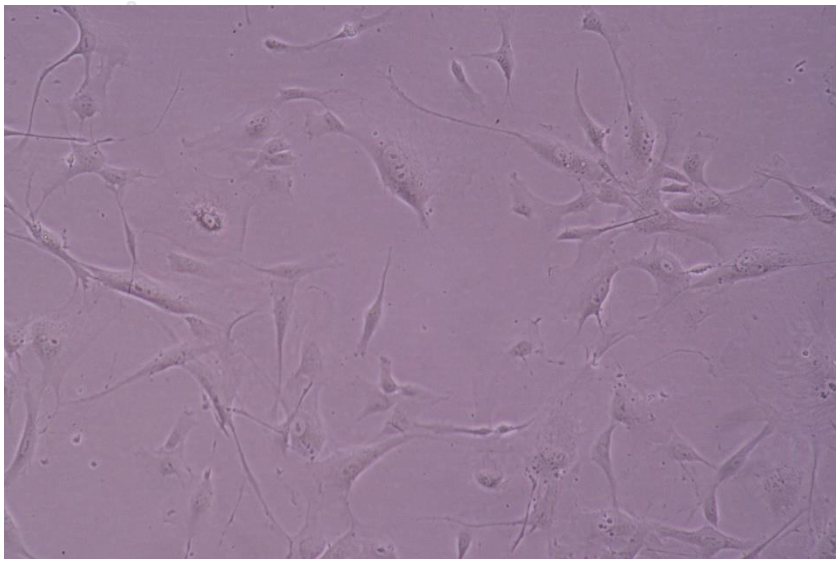
4.2.3 Epithelial-to-Mesenchymal Transition (EMT)

Effluent-derived peritoneal mesothelial cell culture was done to determine the effects of treatments on the EMT of peritoneal mesothelial cells. Cell scores of morphology change could not be measured because of cell culture failure. When peritoneal mesothelial cells from dialysate effluent were cultured, the number of cells was not enough to determine their morphologic classification. Example pictures of peritoneal mesothelial cell culture were shown in Figure 7.

(A)



(B)



(C)

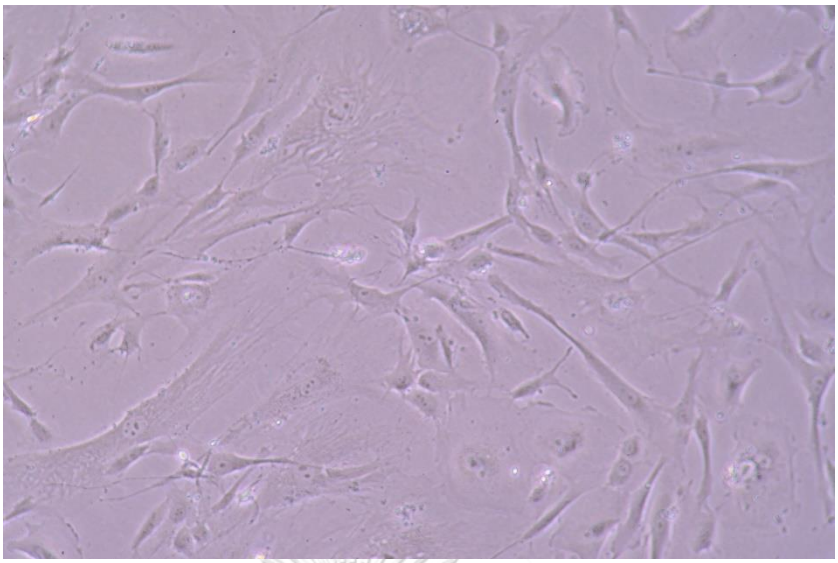


Figure 7 Pictures of peritoneal mesothelial cell culture from patient dialysate effluent in various days of the culture period. (A) 7 days (B) 13 days (C) 20 days

4.3 Adverse events

Patients in sulodexide and placebo group had reported adverse events during the treatment period (Table 7). There was no statistically significant difference between the two groups (p-value = 0.64). The most common adverse event were gastrointestinal discomfort which included flatulence, dyspepsia, nausea, and heartburn. Other adverse events were diarrhea, hair loss, headache, and dizziness. No serious adverse events related or unrelated to sulodexide were observed in both groups. There were 5 patients dropped out from the study (3 patients dropped out from peritonitis and 2 patients dropped out because they could not tolerance to gastrointestinal discomfort and diarrhea). No abnormal Hb, Hct, Plt, aPTT, and PT were reported in both groups.

Table 7 Adverse events in sulodexide and placebo group

Adverse events	Sulodexide (n=33)	Placebo (n=33)
Gastrointestinal discomfort	3	5
Diarrhea	1	1
Hair loss	0	1
Headache	1	0
Dizziness	2	1
Peritonitis	2	1

CHAPTER V

DISCUSSION

Peritoneal dialysis is used by ESRD patients worldwide and the number of patients treated with PD has increased especially in developing countries [103]. Although PD is more cost-effective compared to hemodialysis [104-107], there are limitations in using PD as a long-term treatment. Structural and functional alterations of the peritoneal membrane can occur in long-term PD patients. This study was conducted to investigate the effects of oral sulodexide for the prevention of peritoneal membrane change in CAPD patients. We explored the effect on peritoneal membrane transport, which reflects the functional changes of the peritoneal membrane. Results from 4-hour peritoneal equilibrium test showed that there was a significantly lower D/P creatinine in the sulodexide group than in the placebo group after the treatment period. There are several previous studies reported the contrast results with our study, which D/P creatinine had increased after the administration of sulodexide in CAPD patients [16, 17, 108]. However, there was a difference in research methodology in these previous studies with our study. In their studies, they were uncontrolled clinical trials with a small number of patients and sulodexide was administered by intraperitoneal route except in Fracasso *et al.* study [17], which had oral route of administration. On the contrary, our study found no significant difference in D/D0 glucose between two groups after the treatment period. Fracasso *et al.* reported the same finding that D/D0 glucose value did not change [17]. Indeed, D/P creatinine and D/D0 glucose reflect peritoneal membrane transport status. In long-term PD patients that their peritoneal membrane had deteriorated, there is an increase in small solute transport rate or higher transport status defined by an increase in D/P creatinine and a decrease in D/D0 glucose [24]. An animal model of PD conducted by Pletinck *et al.* indicated that D/P creatinine was increased and D/D0 glucose was decreased in the control group when compared to the

sulodexide group [15]. Therefore, our findings supported that sulodexide contributes to the preservation of peritoneal membrane transport alteration by decreasing D/P creatinine.

To evaluate the effect of sulodexide on D/D0 glucose in patients with and without diabetes mellitus, we performed subgroup analysis by diabetes status at baseline. This subgroup analysis was based on data in a study by Lamb *et al* [109]. They demonstrated that plasma glucose had a significantly positive correlation with D/D0 glucose, therefore, plasma glucose level while performing the PET test maybe a confounding factor. As expected, D/D0 glucose at baseline and after treatment period seemed to be higher in patients with diabetes compared with patients without diabetes. Even though there were no significant differences between D/D0 glucose within each subgroup, in non-diabetes patients, there was a trend that patients in the sulodexide group had a higher change from baseline value than those in the placebo group.

For a 4-hour ultrafiltration volume, our study found that there was a significantly higher volume in the sulodexide group when compared to the placebo group. The same result was also reported in an animal model study [13]. However, there are clinical studies found no significant difference in ultrafiltration volume, which might be due to the difference in research design as mentioned above [16, 17]. Ultrafiltration volume is affected by peritoneal transport function, therefore, higher ultrafiltration volume in the sulodexide group was the result of better peritoneal membrane transport status. The increase in D/P creatinine and decrease in D/D0 glucose indicate that waste toxins pass quickly, classified this type as high transporter. This type will have poor water removal because the water and glucose from the dialysate fluid are absorbed into the body too early and cannot maintain the osmotic gradient [21].

This study showed the tendency of sulodexide in preserving peritoneal membrane function. Because the mechanism of sulodexide is involved in the inhibition

of matrix synthesis and angiogenesis, the permeability of small solutes including creatinine and glucose will consequently decrease and leads to the increase in ultrafiltration volume. Overall, it results in the reduction of volume and uremic toxins retentions.

The levels of dialysate biomarkers were also evaluated in our study. Dialysate CA125 is a biomarker of peritoneal mesothelial cell mass because it is produced by mesothelial cells and can be found in peritoneal dialysate effluent in peritoneal dialysis patients [46]. Measurement of dialysate CA125 can indicate the exfoliation of peritoneal mesothelial cells, which is the early event of membrane structural change. Previous studies found that the longer the duration of PD, the lower the CA125 concentration [49, 50, 52]. This finding is in accordance with our study that there was a significantly lower CA125 concentration from baseline in the placebo group after a 3-month intervention, but in the sulodexide group, there was no significant difference change from baseline. Therefore, Sulodexide may have the potential to inhibit the loss of peritoneal mesothelial cells. To our knowledge, this is the first study to describe the effect of sulodexide on dialysate CA125. The possible mechanism is that sulodexide is believed to reduce TGF-β1, which is a growth factor that contributes to EMT of mesothelial cells [19]. Mesothelial cells that undergo EMT will transform into fibroblast-like cells [20]. So, sulodexide could preserve peritoneal mesothelial cells by this mechanism.

In our study, we also measured dialysate IL-6, which is a marker of inflammation. IL-6 is a proinflammatory cytokine that is locally produced in the peritoneal cavity during PD. Normally, Infectious peritonitis causes an increase in the local production of this cytokine [44]. Our study found a significantly higher level of IL-6 within the placebo group after treatment period while there was no significant difference change within the sulodexide group. Moreover, when compared between groups, higher IL-6 concentration was found in the placebo group. In Fracasso *et al.* study, they also found a statistically significant reduction of IL-6 concentration in the dialysis fluid was induced

by sulodexide [17]. Cross-sectional by Zhou *et al.* reported that dialysate IL-6 concentration was significantly associated with D/P creatinine [110]. However, variable results have been given by several longitudinal studies with regard to the relationship between dialysate IL-6 level and peritoneal membrane transport [44]. Our dialysate IL-6 result was found to be decreased when administered sulodexide means that sulodexide could reduce IL-6, which can lead to matrix synthesis and fibrosis.

In our study, no difference was reported for dialysate VEGF concentration changes both within and between two groups of patients. The same result was found in the animal model study by Pletinck *et al.*, they reported that the difference between control and sulodexide animals did not reach significance [15]. VEGF is Growth factors for angiogenesis and related to peritoneal transport by diffusion of low molecular weight solutes. An increase in VEGF during longitudinal follow-up was shown in a study with a small number of patients, which is in accordance with the progression of neoangiogenesis [44]. However, the negative finding in this study may be explained by the assumption that sulodexide inhibits VEGF activity either by binding it or by inhibiting the interaction with its receptor.

This study also tried to investigate the effect of oral sulodexide on EMT by performing peritoneal mesothelial cells culture isolating from overnight dwell bag of peritoneal dialysis solution, and classify their cell morphology then. Unfortunately, this part of the experiment was not successful because of cell culture problems. When peritoneal mesothelial cells from dialysate effluent were cultured, the number of cells was not enough to determine their morphologic classification. The reason that contribute to cell culture failure may be due to our sedimentation technique. We left the dialysate bag in room temperature for almost 7 hours until we did the centrifugation process. There is a recommendation that the dialysate bag should be hanged in the incubator at 37 ^{0}C for 3 hours. And the other reason was that we put the cell pellets in 25-cm^{2} tissue culture flask, which is too big. They recommended a minimum seeding density of 1-5 x

10^4 cells/cm^2 is required for initial culture from fresh peritoneal dialysate effluent [111, 112].

CHAPTER VI

CONCLUSION

This study investigated the effects of sulodexide for the prevention of peritoneal membrane change in patients undergoing CAPD. It was conducted during 2014 at the Department of Medicine, Phramongkutklao Hospital, and Banphaeo Hospital (Prommitr branch), Bangkok. A total of 66 patients, divided into 33 patients for each group, were included in this randomized control trial study. Patients were randomly assigned to receive either sulodexide or placebo. In the sulodexide group, Patients were assigned to take sulodexide 50 mg 2 times daily before meal for 90 days. In the placebo group, patients were assigned to take placebo, which was similar color and appearance to study drug. Patients took 2 capsules per time, 2 times per day for 90 days. PET with 4-hour dwell of 2 liters of glucose 2.5% PD solution was performed to evaluate peritoneal transport function. At baseline and after the treatment period, peritoneal membrane transport was calculated by D/P of creatinine, D/D0 of glucose. Net ultrafiltration was calculated as the difference between the drained and the instilled volume. Dialysate CA125, IL-6, and VEGF concentration were also measured at baseline and after treatment by ELISA. Peritoneal mesothelial cell culture from dialysate effluent was done to evaluate EMT.

There were 5 patients who dropped out of the study. Overall, 61 patients completed the 3-month study (30 patients in the sulodexide group, 31 patients in the placebo group). Baseline characteristic data found that the two groups were similar for all characteristics. Results of peritoneal transport functions from the per-protocol analysis were reported. After the treatment period, there was a significantly lower D/P creatinine in the sulodexide group than in the placebo group (p-value = 0.04). However, no significant difference in D/D0 glucose was observed between the two groups. For a

4-hour ultrafiltration volume, there was a significantly higher volume in the sulodexide group when compared to the placebo group (p-value = 0.01).

Overnight peritoneal biomarkers changes between two groups were also analyzed. After the treatment period, patients in the sulodexide group had no significant difference change from baseline in peritoneal CA125 concentration while there was a significantly lower CA125 concentration in the placebo group (p-value = 0.03). However, no significant difference in CA125 concentration was found between the two groups. For peritoneal IL-6 concentration, a significantly higher level was found within the placebo group after the treatment period (p-value < 0.01) while there was no significant difference change within the sulodexide group. When compared between groups, a significantly higher IL-6 concentration was found in placebo group than those in the sulodexide group (p-value = 0.03). No significant difference was observed for peritoneal VEGF concentration changes both within and between two groups of patients.

Effluent-derived peritoneal mesothelial cell culture was done to determine the effects of treatments on EMT of peritoneal mesothelial cells. Cell scores of morphology change could not be measured because of cell culture failure. When peritoneal mesothelial cells from dialysate effluent were cultured, the number of cells was not enough to determine their morphologic classification.

In conclusion from overall results in this study, the administration of sulodexide has a potentially beneficial effect in the prevention of peritoneal membrane damage in CAPD patients. Sulodexide may be used to slow the progression of peritoneal membrane change.

Limitations of the present study

1. The result from this study cannot be extrapolated to long-term CAPD patients because we included patients who undergoing CAPD for at least 6 months and had only 3-month treatment period.
2. The duration of treatment was not long enough to detect some peritoneal function test because functional change takes longer time than structural change.
3. There was a small sample size in each group.
4. Peritoneal mesothelial cell culture from dialysate effluent could not be done. The results of the effect on EMT cannot be evaluated.

Recommendations

1. Design study that recruits only long-term CAPD patient.
2. Longer treatment duration should be done to investigate more change of peritoneal membrane function.
3. More sample size should be included to detect the significance of some variables that were not significant in this study.
4. Other methods for evaluating EMT of mesothelial cells should be used such as flow cytometry.

REFERENCES

[1] Grassmann A, Gioberge S, Moeller S, Brown G. ESRD patients in 2004: global overview of patient numbers, treatment modalities and associated trends. Nephrology, dialysis, transplantation 20 (2005): 2587-2593.

[2] Dhanakijcharoen P, Sirivongs D, Aruyapitipan S, Chuengsaman P, Lumpaopong A. The "PD First" policy in Thailand: three-years experiences (2008-2011). Journal of the Medical Association of Thailand 94 Suppl 4 (2011): S153-161.

[3] The Nephrology Society of Thailand. Thailand Renal Replacement Therapy Year 2015 [Available from: http://www.nephrothai.org/TRT/TRT2015/index.html.

[4] Krediet RT. The physiology of peritoneal solute, water, and lymphatic transport. In: Khanna R, Krediet RT, editors. Nolph and Gokal's textbook of peritoneal dialysis. 3 ed. New York: Springer; 2009. p. 137-172.

[5] Schilte MN, Celie JW, Wee PM, Beelen RH, van den Born J. Factors contributing to peritoneal tissue remodeling in peritoneal dialysis. Peritoneal dialysis international 29 (2009): 605-617.

[6] Kaneko K, Hamada C, Tomino Y. Peritoneal fibrosis intervention. Peritoneal dialysis international 27 Suppl 2 (2007): S82-86.

[7] Chaudhary K. Peritoneal dialysis drop-out: causes and prevention strategies. International journal of nephrology 2011 (2011): 1-7.

[8] De Vriese AS, Mortier S, Lameire NH. Non anticoagulant effects of heparin: implications for animal models of peritoneal dialysis. Peritoneal dialysis international 21 Suppl 3 (2001): S354-356.

[9] Margetts P. Heparin and the peritoneal membrane. Peritoneal dialysis international 29 (2009): 16-19.

[10] Farooq V, Hegarty J, Chandrasekar T, Lamerton EH, Mitra S, Houghton JB, et al. Serious adverse incidents with the usage of low molecular weight heparins in patients with chronic kidney disease. American journal of kidney diseases 43 (2004): 531-537.

[11] Harenberg J. Review of pharmacodynamics, pharmacokinetics, and therapeutic

properties of sulodexide. Medicinal research reviews 18 (1998): 1-20.

[12] Lauver DA, Lucchesi BR. Sulodexide: a renewed interest in this glycosaminoglycan. Cardiovascular drug reviews 24 (2006): 214-226.

[13] Fracasso A, Baggio B, Ossi E, Del Prete D, Bonfante L, Bazzato G, et al. Glycosaminoglycans prevent the functional and morphological peritoneal derangement in an experimental model of peritoneal fibrosis. American journal of kidney diseases 33 (1999): 105-110.

[14] Karon J, Polubinska A, Antoniewicz AA, Suminska-Jasinska K, Breborowicz A. Anti-inflammatory effect of sulodexide during acute peritonitis in rats. Blood purification 25 (2007): 510-514.

[15] Pletinck A, Van Landschoot M, Steppan S, Laukens D, Passlick-Deetjen J, Vanholder R, et al. Oral supplementation with sulodexide inhibits neo-angiogenesis in a rat model of peritoneal perfusion. Nephrology, dialysis, transplantation 27 (2012): 548-556.

[16] Bazzato G, Fracasso A, Gambaro G, Baggio B. Use of glycosaminoglycans to increase efficiency of long-term continuous peritoneal dialysis. Lancet 346 (1995): 740-741.

[17] Fracasso A, Baggio B, Masiero M, Bonfante L, Bazzato G, Feriani M, et al. Effect of oral treatment with the glycosaminoglycan sulodexide on peritoneal transport in CAPD patients. Peritoneal dialysis international 23 (2003): 595-599.

[18] Gotloib L. Functional structure of the peritoneum as a dialyzing membrane. In: Khanna R, Krediet RT, editors. Nolph and Gokal's textbook of peritoneal dialysis. 3 ed. New York: Springer; 2009. p. 73-135.

[19] Baroni G, Schuinski A, de Moraes TP, Meyer F, Pecoits-Filho R. Inflammation and the peritoneal membrane: causes and impact on structure and function during peritoneal dialysis. Mediators of inflammation 2012 (2012): 912595.

[20] Yung S, Chan T-M. Peritoneal fibrosis-future prevention strategies. Hong Kong Journal of Nephrology 5 (2003): 8-14.

[21] Devuyst O, Westrhenen RV, Topley N. Long-term peritoneal dialysis patients: changes in membrane structure and function. In: Khanna R, Krediet RT, editors. Nolph and

Gokal's textbook of peritoneal dialysis. 3 ed. New York: Springer; 2009. p. 757-780.

[22] Plum J, Hermann S, Fussholler A, Schoenicke G, Donner A, Röhrborn A, et al. Peritoneal sclerosis in peritoneal dialysis patients related to dialysis settings and peritoneal transport properties. Kidney International 59 (2001): S42-S47.

[23] Yáñez-Mó M, Lara-Pezzi E, Selgas R, Ramírez-Huesca M, Domínguez-Jiménez C, Jiménez-Heffernan JA, et al. Peritoneal dialysis and epithelial-to-mesenchymal transition of mesothelial cells. New England Journal of Medicine 348 (2003): 403-413.

[24] Goffin E. Peritoneal membrane structural and functional changes during peritoneal dialysis. Seminars in dialysis 21 (2008): 258-265.

[25] Devuyst O. New insights in the molecular mechanisms regulating peritoneal permeability. Nephrology, dialysis, transplantation 17 (2002): 548-551.

[26] Mortier S, De Vriese AS, Lameire N. Recent concepts in the molecular biology of the peritoneal membrane - implications for more biocompatible dialysis solutions. Blood purification 21 (2003): 14-23.

[27] Williams JD, Craig KJ, Topley N, Von Ruhland C, Fallon M, Newman GR, et al. Morphologic changes in the peritoneal membrane of patients with renal disease. Journal of the American Society of Nephrology 13 (2002): 470-479.

[28] Davies SJ, Phillips L, Griffiths AM, Russell LH, Naish PF, Russell GI. Impact of peritoneal membrane function on long-term clinical outcome in peritoneal dialysis patients. Peritoneal dialysis international 19 Suppl 2 (1999): S91-94.

[29] Davies SJ, Mushahar L, Yu Z, Lambie M. Determinants of peritoneal membrane function over time. Seminars in nephrology 31 (2011): 172-182.

[30] Breborowicz A, Oreopoulos DG. Physiological approaches to increase biocompatibility of peritoneal dialysis. Peritoneal dialysis international 15 (1995): S76-85; discussion S85-76.

[31] Heimburger O, Waniewski J, Werynski A, Lindholm B. A quantitative description of solute and fluid transport during peritoneal dialysis. Kidney International 41 (1992): 1320-1332.

[32] Tzamaloukas AH. Peritoneal toxicities of hypertonic dextrose dialysate. Advances

in peritoneal dialysis 15 (1999): 217-221.

[33] Nakayama M, Kawaguchi Y, Yamada K, Hasegawa T, Takazoe K, Katoh N, et al. Immunohistochemical detection of advanced glycosylation end-products in the peritoneum and its possible pathophysiological role in CAPD. Kidney International 51 (1997): 182-186.

[34] Honda K, Nitta K, Horita S, Yumura W, Nihei H, Nagai R, et al. Accumulation of advanced glycation end products in the peritoneal vasculature of continuous ambulatory peritoneal dialysis patients with low ultra-filtration. Nephrology, dialysis, transplantation 14 (1999): 1541-1549.

[35] Dobbie JW, Lloyd JK, Gall CA. Categorization of ultrastructural changes in peritoneal mesothelium, stroma and blood vessels in uremia and CAPD patients. Advances in peritoneal dialysis 6 (1990): 3-12.

[36] Wong TY, Phillips AO, Witowski J, Topley N. Glucose-mediated induction of TGF-beta 1 and MCP-1 in mesothelial cells in vitro is osmolality and polyol pathway dependent. Kidney International 63 (2003): 1404-1416.

[37] Ing TS, Yu AW, Podila PV, Zhou FQ, Kun EW, Strippoli P, et al. Failure of neutrophils to recover their ability to produce superoxide after stunning by a conventional, acidic, lactate-based peritoneal dialysis solution. The International journal of artificial organs 17 (1994): 191-194.

[38] Struijk DG, Khanna R. Monitoring the fuctional status of the peritoneum. In: Khanna R, Krediet RT, editors. Nolph and Gokal's textbook of peritoneal dialysis. 3 ed. New York: Springer; 2009. p. 447-468.

[39] Wolf CJ, Polsky J, Ntoso KA, Koethe JD, Gerhardt RE. Adequacy of dialysis in CAPD and cycler PD; the PET is enough. Advances in peritoneal dialysis 8 (1992): 208-211.

[40] Twardowski Zj, Nolph KO, Khanna R, Prowant BF, Ryan LP, Moore HL, et al. Peritoneal equilibration test. Peritoneal dialysis international 7 (1987): 138-148.

[41] Kanjanabuch T. Basic and advanced monitoring in peritoneal dialysis. In: Eiam-Ong S, editor. Clinical dialysis. 1 ed. Bangkok: Text and Jounal publication; 2008. p. 1059-1083.

[42] Fresenius Medical Care North America. Transport Status: Classification and Clinical Implications [Available from: http://www.advancedrenaleducation.com/PeritonealDialysis/PeritonealTransport/TransportStatusClassificationandImplications/tabid/81/Default.aspx.

[43] National Kidney Foundation. Clinical Practice Guidelines for Peritoneal Dialysis Adequacy. American journal of kidney diseases 48 (2006): S98-S129.

[44] Krediet RT, Struijk DG. Peritoneal changes in patients on long-term peritoneal dialysis. Nature Reviews Nephrology 9 (2013): 419-429.

[45] Krediet RT. Dialysate cancer antigen 125 concentration as marker of peritoneal membrane status in patients treated with chronic peritoneal dialysis. Peritoneal dialysis international 21 (2001): 560-567.

[46] Koomen GC, Betjes MG, Zemel D, Krediet RT, Hoek FJ. Cancer antigen 125 is locally produced in the peritoneal cavity during continuous ambulatory peritoneal dialysis. Peritoneal dialysis international 14 (1994): 132-136.

[47] Visser CE, Brouwer-Steenbergen JJ, Betjes MG, Koomen GC, Beelen RH, Krediet RT. Cancer antigen 125: a bulk marker for the mesothelial mass in stable peritoneal dialysis patients. Nephrology, dialysis, transplantation 10 (1995): 64-69.

[48] Ho-dac-Pannekeet MM, Hiralall JK, Struijk DG, Krediet RT. Longitudinal follow-up of CA125 in peritoneal effluent. Kidney International 51 (1997): 888-893.

[49] Passadakis P, Panagoutsos S, Thodis E, Tsivara I, Sopassi F, Kartali S, et al. Evaluation of changes in serum and dialysate levels of cancer antigen 125 in stable continuous ambulatory peritoneal dialysis patients. Advances in peritoneal dialysis 15 (1999): 40-44.

[50] Lopes Barreto D, Coester AM, Noordzij M, Smit W, Struijk DG, Rogers S, et al. Variability of effluent cancer antigen 125 and interleukin-6 determination in peritoneal dialysis patients. Nephrology, dialysis, transplantation 26 (2011): 3739-3744.

[51] Jimenez C, Diaz C, Selgas R, Auxiliadora Bajo M, del Peso G, Sanchez-Tomero JA, et al. Peritoneal kinetics of cancer antigen 125 in peritoneal dialysis patients: the relationship with peritoneal outcome. Advances in peritoneal dialysis 15 (1999): 36-39.

[52] Bouts AH, Groothoff JW, van Amstel SP, Zweers MM, Davin JC, Krediet RT. Dialysate cancer antigen 125 levels in children treated with peritoneal dialysis. Advances in peritoneal dialysis 16 (2000): 328-331.

[53] le Poole CY, Welten AG, ter Wee PM, Paauw NJ, Djorai AN, Valentijn RM, et al. A peritoneal dialysis regimen low in glucose and glucose degradation products results in increased cancer antigen 125 and peritoneal activation. Peritoneal dialysis international 32 (2012): 305-315.

[54] Garcia H, Hernandez-Jaras J, Cruz Mdel C, Agramunt I, Calvo C, Cerrillo V. Short- and medium-term increase of CA125 in peritoneal effluent using a neutral-pH solution. Peritoneal dialysis international 23 (2003): 375-380.

[55] Choi HY, Kim DK, Lee TH, Moon SJ, Han SH, Lee JE, et al. The clinical usefulness of peritoneal dialysis fluids with neutral pH and low glucose degradation product concentration: an open randomized prospective trial. Peritoneal dialysis international 28 (2008): 174-182.

[56] Theodoridis M, Passadakis P, Kriki P, Gioka T, Panagoutsos S, Mourvati E, et al. The alteration of dialysate cancer antigen 125 concentration under a biocompatible bicarbonate peritoneal dialysis solution and the preservation of the mesothelial cell viability. Renal failure 30 (2008): 161-167.

[57] Sampimon DE, Korte MR, Barreto DL, Vlijm A, de Waart R, Struijk DG, et al. Early diagnostic markers for encapsulating peritoneal sclerosis: a case-control study. Peritoneal dialysis international 30 (2010): 163-169.

[58] Williams JD, Topley N, Craig KJ, Mackenzie RK, Pischetsrieder M, Lage C, et al. The Euro-Balance Trial: the effect of a new biocompatible peritoneal dialysis fluid (balance) on the peritoneal membrane. Kidney International 66 (2004): 408-418.

[59] Bajo MA, Perez-Lozano ML, Albar-Vizcaino P, del Peso G, Castro MJ, Gonzalez-Mateo G, et al. Low-GDP peritoneal dialysis fluid ('balance') has less impact in vitro and ex vivo on epithelial-to-mesenchymal transition (EMT) of mesothelial cells than a standard fluid. Nephrology, dialysis, transplantation 26 (2011): 282-291.

[60] Johnson DW, Brown FG, Clarke M, Boudville N, Elias TJ, Foo MW, et al. The effect

of low glucose degradation product, neutral pH versus standard peritoneal dialysis solutions on peritoneal membrane function: the balANZ trial. Nephrology, dialysis, transplantation 27 (2012): 4445-4453.

[61] Johnson DW, Brown FG, Clarke M, Boudville N, Elias TJ, Foo MW, et al. Effects of biocompatible versus standard fluid on peritoneal dialysis outcomes. Journal of the American Society of Nephrology 23 (2012): 1097-1107.

[62] Cho Y, Johnson DW, Craig JC, Strippoli GF, Badve SV, Wiggins KJ. Biocompatible dialysis fluids for peritoneal dialysis. The Cochrane database of systematic reviews (2014): Cd007554.

[63] Seo EY, An SH, Cho JH, Suh HS, Park SH, Gwak H, et al. Effect of biocompatible peritoneal dialysis solution on residual renal function: a systematic review of randomized controlled trials. Peritoneal dialysis international 34 (2014): 724-731.

[64] Bruno M, Bagnis C, Marangella M, Rovera L, Cantaluppi A, Linari F. CAPD with an amino acid dialysis solution: a long-term, cross-over study. Kidney International 35 (1989): 1189-1194.

[65] le Poole CY, Welten AG, Weijmer MC, Valentijn RM, van Ittersum FJ, ter Wee PM. Initiating CAPD with a regimen low in glucose and glucose degradation products, with icodextrin and amino acids (NEPP) is safe and efficacious. Peritoneal dialysis international 25 Suppl 3 (2005): S64-68.

[66] García-López E, Lindholm B, Davies S. An update on peritoneal dialysis solutions. Nature Reviews Nephrology 8 (2012): 224-233.

[67] Johnson DW, Agar J, Collins J, Disney A, Harris DC, Ibels L, et al. Recommendations for the use of icodextrin in peritoneal dialysis patients. Nephrology (Carlton) 8 (2003): 1-7.

[68] Mistry CD, Gokal R, Peers E. A randomized multicenter clinical trial comparing isosmolar icodextrin with hyperosmolar glucose solutions in CAPD. MIDAS Study Group. Multicenter Investigation of Icodextrin in Ambulatory Peritoneal Dialysis. Kidney International 46 (1994): 496-503.

[69] Garcia-Lopez E, Lindholm B. Icodextrin metabolites in peritoneal dialysis.

Peritoneal dialysis international 29 (2009): 370-376.

[70] Bajo MA, Selgas R, Castro MA, del Peso G, Diaz C, Sanchez-Tomero JA, et al. Icodextrin effluent leads to a greater proliferation than glucose effluent of human mesothelial cells studied ex vivo. Peritoneal dialysis international 20 (2000): 742-747.

[71] Davies SJ, Woodrow G, Donovan K, Plum J, Williams P, Johansson AC, et al. Icodextrin improves the fluid status of peritoneal dialysis patients: results of a double-blind randomized controlled trial. Journal of the American Society of Nephrology 14 (2003): 2338-2344.

[72] Lin A, Qian J, Li X, Yu X, Liu W, Sun Y, et al. Randomized controlled trial of icodextrin versus glucose containing peritoneal dialysis fluid. Clinical journal of the American Society of Nephrology 4 (2009): 1799-1804.

[73] Plum J, Gentile S, Verger C, Brunkhorst R, Bahner U, Faller B, et al. Efficacy and safety of a 7.5% icodextrin peritoneal dialysis solution in patients treated with automated peritoneal dialysis. American journal of kidney diseases 39 (2002): 862-871.

[74] Takatori Y, Akagi S, Sugiyama H, Inoue J, Kojo S, Morinaga H, et al. Icodextrin increases technique survival rate in peritoneal dialysis patients with diabetic nephropathy by improving body fluid management: a randomized controlled trial. Clinical journal of the American Society of Nephrology 6 (2011): 1337-1344.

[75] Yoon HE, Chang YK, Shin SJ, Choi BS, Kim BS, Park CW, et al. Benefits of a continuous ambulatory peritoneal dialysis (CAPD) technique with one icodextrin-containing and two biocompatible glucose-containing dialysates for preservation of residual renal function and biocompatibility in incident CAPD patients. Journal of Korean medical science 29 (2014): 1217-1225.

[76] Border WA, Noble NA. TGF-beta in kidney fibrosis: a target for gene therapy. Kidney International 51 (1997): 1388-1396.

[77] Yao Q, Qian JQ, Lin XH, Lindholm B. Inhibition of the effect of high glucose on the expression of Smad in human peritoneal mesothelial cells. The International journal of artificial organs 27 (2004): 828-834.

[78] Subeq YM, Ke CY, Lin NT, Lee CJ, Chiu YH, Hsu BG. Valsartan decreases TGF-

beta1 production and protects against chlorhexidine digluconate-induced liver peritoneal fibrosis in rats. Cytokine 53 (2011): 223-230.

[79] Duman S, Sen S, Duman C, Oreopoulos DG. Effect of valsartan versus lisinopril on peritoneal sclerosis in rats. The International journal of artificial organs 28 (2005): 156-163.

[80] Duman S, Wieczorowska-Tobis K, Styszynski A, Kwiatkowska B, Breborowicz A, Oreopoulos DG. Intraperitoneal enalapril ameliorates morphologic changes induced by hypertonic peritoneal dialysis solutions in rat peritoneum. Advances in peritoneal dialysis 20 (2004): 31-36.

[81] Perez-Martinez J, Perez-Martinez FC, Carrion B, Masia J, Ortega A, Simarro E, et al. Aliskiren prevents the toxic effects of peritoneal dialysis fluids during chronic dialysis in rats. PLoS One 7 (2012): e36268.

[82] Ke CY, Lee CC, Lee CJ, Subeq YM, Lee RP, Hsu BG. Aliskiren ameliorates chlorhexidine digluconate-induced peritoneal fibrosis in rats. European journal of clinical investigation 40 (2010): 301-309.

[83] Nishimura H, Ito Y, Mizuno M, Tanaka A, Morita Y, Maruyama S, et al. Mineralocorticoid receptor blockade ameliorates peritoneal fibrosis in new rat peritonitis model. American journal of physiology 294 (2008): F1084-1093.

[84] Kolesnyk I, Dekker FW, Noordzij M, le Cessie S, Struijk DG, Krediet RT. Impact of ACE inhibitors and AII receptor blockers on peritoneal membrane transport characteristics in long-term peritoneal dialysis patients. Peritoneal dialysis international 27 (2007): 446-453.

[85] Jing S, Kezhou Y, Hong Z, Qun W, Rong W. Effect of renin-angiotensin system inhibitors on prevention of peritoneal fibrosis in peritoneal dialysis patients. Nephrology (Carlton) 15 (2010): 27-32.

[86] Trost Rupnik A, Pajek J, Gucek A, Osredkar J, Kovac D, Bren A, et al. Influence of renin-angiotensin-aldosterone system-blocking drugs on peritoneal membrane in peritoneal dialysis patients. Therapeutic apheresis and dialysis 17 (2013): 425-430.

[87] Kolesnyk I, Noordzij M, Dekker FW, Boeschoten EW, Krediet RT. A positive effect of AII inhibitors on peritoneal membrane function in long-term PD patients. Nephrology,

dialysis, transplantation 24 (2009): 272-277.

[88] Yelken B, Gorgulu N, Gursu M, Yazici H, Caliskan Y, Telci A, et al. Effects of spironolactone on residual renal function and peritoneal function in peritoneal dialysis patients. Advances in peritoneal dialysis 30 (2014): 5-10.

[89] Ito Y, Mizuno M, Suzuki Y, Tamai H, Hiramatsu T, Ohashi H, et al. Long-term effects of spironolactone in peritoneal dialysis patients. Journal of the American Society of Nephrology 25 (2014): 1094-1102.

[90] Sheriff DS. Medical biochemistry. 1 ed. New Delhi: Jaypee Brothers Publishers; 2004.

[91] Fraser DJ, Topley N. Altering peritoneal membrane function: removing the GAG? Nephrology, dialysis, transplantation 24 (2009): 3271-3273.

[92] Yung S, Chan TM. Pathophysiology of the peritoneal membrane during peritoneal dialysis: the role of hyaluronan. Journal of biomedicine & biotechnology 2011 (2011): 180594.

[93] Breborowicz A, Pyda M, Moberly J, Martis L, Oreopoulos D. Effect of hyaluronan-supplemented dialysate on in vitro function of human peritoneal mesothelial cells. American journal of nephrology 24 (2004): 316-321.

[94] Suyama K, Kumano K, Sasaki T. [Agents preventing decrease in ultrafiltration in a rat model with peritoneal hyperpermeability]. Nihon Jinzo Gakkai Shi 37 (1995): 491-496.

[95] Bazargani F, Albrektsson A, Yahyapour N, Braide M. Low molecular weight heparin improves peritoneal ultrafiltration and blocks complement and coagulation. Peritoneal dialysis international 25 (2005): 394-404.

[96] Sjoland JA, Smith Pedersen R, Jespersen J, Gram J. Intraperitoneal heparin reduces peritoneal permeability and increases ultrafiltration in peritoneal dialysis patients. Nephrology, dialysis, transplantation 19 (2004): 1264-1268.

[97] Li PK, Szeto CC, Piraino B, de Arteaga J, Fan S, Figueiredo AE, et al. ISPD Peritonitis Recommendations: 2016 Update on Prevention and Treatment. Peritoneal dialysis international 36 (2016): 481-508.

[98] Lasierra-Cirujeda J, Coronel P, Aza M, Gimeno M. Use of sulodexide in patients

with peripheral vascular disease. Journal of blood medicine 1 (2010): 105-115.

[99] Wiedermann Ch J, Romisch J. The anti-inflammatory actions of antithrombin--a review. Acta medica Austriaca 29 (2002): 89-92.

[100] Sjoland JA, Pedersen RS, Jespersen J, Gram J. Intraperitoneal heparin ameliorates the systemic inflammatory response in PD patients. Nephron Clinical Practice 100 (2005): c105-110.

[101] Khunprakant R. Role of thiazolidinediones to insulin sensitivity and against peritoneal membrane dysfunction in capd patients [Master of Science's thesis]. Bangkok: Chulalongkorn University; 2007.

[102] Fusshoeller A, Plail M, Grabensee B, Plum J. Biocompatibility pattern of a bicarbonate/lactate-buffered peritoneal dialysis fluid in APD: a prospective, randomized study. Nephrology, dialysis, transplantation 19 (2004): 2101-2106.

[103] Jain AK, Blake P, Cordy P, Garg AX. Global trends in rates of peritoneal dialysis. Journal of the American Society of Nephrology 23 (2012): 533-544.

[104] Karopadi AN, Mason G, Rettore E, Ronco C. Cost of peritoneal dialysis and haemodialysis across the world. Nephrology, dialysis, transplantation 28 (2013): 2553-2569.

[105] Liu F, Quock T, Burkart J, Noe L, Inglese G. Economic evaluations of peritoneal dialysis and hemodialysis: 2004-2012 F1000Research 2 (2013).

[106] Mehrotra R, Chiu YW, Kalantar-Zadeh K, Bargman J, Vonesh E. Similar outcomes with hemodialysis and peritoneal dialysis in patients with end-stage renal disease. Archives of internal medicine 171 (2011): 110-118.

[107] Treharne C, Liu F, Arici M, Crowe L, Farooqui U. Peritoneal Dialysis and In-Centre Haemodialysis: A Cost-Utility Analysis from a UK Payer Perspective. Applied health economics and health policy (2014): 409-420.

[108] Guedri Y, Damma KN, Toumi M, Sahtout W, Azzabi A, Mrabet S, et al. Effect of intraperitoneal injection of sulodexide on peritoneal function and albumin leakage in continuous ambulatory peritoneal dialysis patients. Saudi journal of kidney diseases and transplantation 27 (2016): 1194-1199.

[109] Lamb EJ, Worrall J, Buhler R, Harwood S, Cattell WR, Dawnay AB. Effect of diabetes and peritonitis on the peritoneal equilibration test. Kidney International 47 (1995): 1760-1767.

[110] Zhou L, Wen F, Chen G, Liu J, Liu H, Peng Y, et al. Cytokine profiles in peritoneal dialysis effluent predicts the peritoneal solute transport rate in continuous ambulatory peritoneal dialysis patients. International journal of clinical and experimental medicine 8 (2015): 20424-20433.

[111] Chen KS, Chen WS. Experience in primary culture of human peritoneal mesothelial cell. The Chinese journal of physiology 55 (2012): 274-283.

[112] Yung S, Li FK, Chan TM. Peritoneal mesothelial cell culture and biology. Peritoneal dialysis international 26 (2006): 162-173.

APPENDICES

Appendix A

Ethical Approval Documents

RL 01_2556

ที่ IRB/RTA 012 /2557

คณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบก
317 ถนนราชวิถี เขต ราชเทวี กรุงเทพฯ 10400

รหัสโครงการ: Q022hh/55

ชื่อโครงการวิจัย : "ผลของยาซูโลเดกไซด์ในการป้องกันภาวะเยื่อบุช่องท้องเสื่อมที่ได้รับการล้างไตทางช่องท้อง"
[Effect of sulodexide for the prevention of peritoneal membrane dysfunction in continuous ambulatory peritoneal dialysis patients.]

เลขที่โครงการวิจัย : -

ชื่อผู้วิจัยหลัก: ภญ.ปณิดา ติศรวนนท์

สังกัดหน่วยงาน : ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

สถานที่ทำการวิจัย: โรงพยาบาลพระมงกุฎเกล้า และโรงพยาบาลบ้านแพ้ว (องค์กรมหาชน)

เอกสารรับรอง :

(1) โครงร่างการวิจัยฉบับภาษาไทย ฉบับที่ 2 วันที่ 20 ธันวาคม 2556
(2) เอกสารชี้แจงข้อมูลแก่ผู้เข้าร่วมโครงการวิจัย ฉบับที่ 2 วันที่ 20 ธันวาคม 2556
(3) หนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย ฉบับที่ 2 วันที่ 20 ธันวาคม 2556
(4) แบบบันทึกข้อมูลลงทะเบียน ฉบับที่ 2 วันที่ 20 ธันวาคม 2556
(5) แบบบันทึกข้อมูลตรวจติดตาม ฉบับที่ 2 วันที่ 20 ธันวาคม 2556
(6) แบบบันทึกผล Kt/V, CCr, PET ฉบับที่ 2 วันที่ 20 ธันวาคม 2556
(7) แบบบันทึกผล CA125, IL-6, VEGF, Exfoliated mesothelial cells ฉบับที่ 2 วันที่ 20 ธันวาคม 2556
(8) แบบบันทึกข้อมูลโดยผู้ป่วย ฉบับที่ 2 วันที่ 20 ธันวาคม 2556
(9) แบบประเมินความสัมพันธ์ระหว่างอาการไม่พึงประสงค์กับยาที่สงสัย ฉบับที่ ฉบับที่ 2 วันที่ 20 ธันวาคม 2556
(10) ประวัติย่อ ภญ.ปณิดา ติศรวนนท์ ฉบับที่ 1 วันที่ 25 ตุลาคม 2556
(11) ประวัติย่อ รศ.ภญ.ดร.พรอนงค์ อร่ามวิทย์ ฉบับที่ 1 วันที่ 25 ตุลาคม 2556
(12) ประวัติย่อ พ.ต.อุปถัมภ์ ศุภสินธุ์ ฉบับที่ 1 วันที่ 25 ตุลาคม 2556

ขอรับรองว่าโครงการดังกล่าวข้างต้นได้ผ่านการพิจารณารับรองจากคณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบก ว่าสอดคล้องกับปฏิญญาเฮลซิงกิ และแนวปฏิบัติ ICH GCP

วันที่รับรองด้านจริยธรรมของโครงร่างการวิจัย: 27 ธันวาคม 2556
วันสิ้นสุดการรับรอง: 26 ธันวาคม 2557
ความถี่ของการส่งรายงานความก้าวหน้าของการวิจัย: รายงานความก้าวหน้าทุก 1 ปี

พันเอกหญิง เสาวนา ชนะศัตรู
ประธานคณะอนุกรรมการพิจารณาโครงการวิจัย พบ.

พันเอกสมพล อนันต์น่าเจริญ
เลขานุการและอนุกรรมการพิจารณาโครงการวิจัย พบ.

Appendix B

Registration record form

Date of register : Date_____ Month_____ Year_____

1. Code No._____
2. Age _____
3. Gender ☐ 1. Male ☐ 2. Female
4. Education level
   - ☐ 1. None ☐ 2. Elementary
   - ☐ 3. Junior High School ☐ 4. Senior High School
   - ☐ 5. College ☐ 6. Bachelor's degree and higher
5. Healthcare services
   - ☐ 1. Direct payment from government ☐ 2. Social Security
   - ☐ 3. Universal Health Coverage ☐ 4. State Enterprises
   - ☐ 5. Other_____
6. Cause of ESRD
   - ☐ 1. MN ☐ 2. LN ☐ 3.DN ☐ 4.IgMN
   - ☐ 5. FSGS ☐ 6. MPGN ☐ 7.IgAN ☐ 8. PSGN
   - ☐ 9.Obstruction ☐ 10. Ischemia ☐ 11.Unknown ☐ 12.อื่นๆ_____
9. Co-morbid disease
   - ☐ 1. DM ☐ 2. HT ☐ 3.DLP ☐ 4.CVD(IHD,CHF,CABG)
   - ☐ 5. COPD ☐ 6.AF ☐ 7.HIV ☐ 8.CVA
   - ☐ 9.PVD ☐ 10. CHB/HCV ☐ 11.Other _____
10. Tenckhoff catheter placement date _____/_____/_____

    Peritoneal dialysis start date _____/_____/_____

    Timing start PD until register (mo) ☐☐
11. Peritoneal dialysis dose
    - ☐ 1. Normal dose (8-10 L.) ☐ 2. High dose (>10 L.)

12. Type of peritoneal fluid glucose/dextrose

☐	1. Baxter	☐ 1. 1.36% Number of bags/day _____
		☐ 2. 2.27% Number of bags/day _____
		☐ 3. 3.86% Number of bags/day _____
☐	2. Fresineus	☐ 1. 1.5% Number of bags/day _____
		☐ 2. 2.3% Number of bags/day _____
		☐ 3. 4.25% Number of bags/day _____

13. Net ultrafiltration (per day) _____

14. Urine output (per day) _____

15. Physical examination : Hight _____ cm Weight _____ kg

Vital signs : BP _____ mmHg Pulse _____ bpm

Abdomen : ☐ Normal ☐ Abnormal _____

☐ Hernia

☐ Surgical scar

Edema : ☐ No ☐ Yes _____

16. Current medications (Name/Strength, Administration, Indication, Start-Stop date)

Appendix C

Monitoring record form

Code No._____

Visiting date : Date_____ Month_____ Year_____

No. of visit _____

Adherence

1. Reason for postpone an appointment

☐ 1. On time for appointment ☐ 2. Forgot appointment ☐ 3. Personal matter

☐ 4. Others

2. Frequency of missing to take medicine

☐ 1. never ☐ 2. 2-3 times

☐ 3. 4-5 times ☐ 4. ≥ 6 times

☐ 5. > 2 weeks

2. How to do when missing a dose?

☐ 1. Take it as soon as remember ☐ 2. Skip the missed dose

☐ 3. Take extra dose in the next scheduled dose ☐ 4. Other_____

3. Number of receive drug _____ tablets

4. Number of remain drug tablets

$$\frac{\text{No. of Pills Absent in Time}}{\text{No. of Pills Prescribed for Time}} \times 100 = \_\_\_\_\_ \%$$

Adverse effect

☐ Nausea

☐ Vomiting

☐ Diarrhea

☐ Chest pain

☐ Rash

☐ Peritonitis

☐ other_____

Peritoneal dialysis information

1. Peritoneal dialysis dose

☐ 1. normal dose (8-10 L.) ☐ 2. High dose (>10 L.)

2. Overnight peritoneal dialysis duration (hours) _____

3. Dialysate drainvolume (mL.) _____

4. Type of peritoneal fluid glucose/dextrose

☐ 1. Baxter
- ☐ 1. 1.36% Number of bags/day _____
- ☐ 2. 2.27% Number of bags/day _____
- ☐ 3. 3.86% Number of bags/day _____

☐ 2. Fresineus
- ☐ 1. 1.5% Number of bags/day _____
- ☐ 2. 2.3% Number of bags/day _____
- ☐ 3. 4.25% Number of bags/day _____

15. Net ultrafiltration (per day) _____

16. Urine output (per day) _____

17. Physical examination :Hight _____ cm Weight _____ kg

Vital signs : BP _____ mmHg Pulse _____ bpm

Abdomen : ☐ Normal ☐ Abnormal _____

☐ Hernia

☐ Surgical scar

Edema : ☐ No ☐ Yes _____

18. Current medications (Name/Strength, Administration, Indication, Start-Stop date)

Laboratory

Date _____

Hematology								
Hb	Hct	PLT	aPTT	PT				
Blood Chemistry								
BUN	Cr	Na	K	Cl	HCO_3	Ca	Mg	PO_4
Liver function								
AST	ALT	ALP	ALB					
Urine chemistry								
UVol								
Dialysate analysis								
Dvol	Dglu	Dcr	DAlb	Kt/V				

Prescription

1. Early withdrawal

☐ 0. No ☐ 1. Lost to follow up ☐ 2. Withdraw consent

☐ 3. Adverse effect ☐ 4. Technical failure ☐ 5. Peritonitis

☐ 6. Death ☐ 7. Other _____

2. Dialysis

☐ 1. Not adjust ☐ 2. Adjusted _____

Follow up date : Date_____ Month_____ Year_____

Peritoneal Equilibration Test record

Code No._____

No. test _____: Date_____ Month_____ Year_____

Sr Cr _____ mg/dl, BUN _____ mg/dl

urine Cr _____ mg/dl, UUN _____ mg/dl, protein _____ g/dl (from 24 hr urine collection)

dialysate Cr ____ mg/dl, dialysate urea _____ mg/dl, protein _____ g/dl (from 24 hr dialysate drain collection)

dialysate Cr : at 2nd hour_____ mg/dl, at 4th hour _____ mg/dl

dialysate glucose : at 2nd hour_____ mg/dl, at 4th hour _____ mg/dl

residual urine volume _____ ml/day = _____ L/day

dialysate drain volume _____ ml/day = _____ L/day

1. **Weekly Kt/v**

dailyKt/V urea = _____weekly Kt/V urea = _____

2. **Weekly CCr**

dailyCCr = _____ weekly CCr = _____ = _____

L/week/1.73 m^2

3. **PET**

D/D_0 glucose (0 hr) = _____ D/P creatinine (0 hr) = _____

D/D_0 glucose (2 hr) = _____ D/P creatinine (2 hr) = _____

D/D_0 glucose (4 hr) = _____ D/P creatinine (4 hr) = _____

Ultrafiltration volume = _____ ml.

Conclusion type of peritoneal membrane

☐ low ☐ low average ☐ high average ☐ high

4. **Interpretation CAPD adequacy**

☐ Adequate ☐ inadequate by weekly Kt/V urea criteria

☐ Adequate ☐ inadequate by weekly CCr/BSA 1.73 m^2 criteria

Appendix D

Self-applied recorded form

Code No._____ No. of visit _____

1. Adverse events record

Adverse event	Symptom	Duration (Start-Stop date)	Treatment	Note
Nausea				
Vomiting				
Diarrhea				
Chest pain				
Rash				
Bleeding				
Bruise				
Other.....				
Other.....				

2. Peritoneal fluid intake and output record

Date	Output - Intake	Solution characteristics

Appendix E

Enzyme Linked Immunosorbent Assay Kit (Bio-Sciences, USA)

Plate preparation

1. Coat 96-well microplate overnight with 1 µg/ml (0.1 µg per well) of antibody, diluted in 0.006 M Carbonate buffer, pH 9.6. Incubate the plate for 24 hours at 4°C.
2. Wash the plate three times with 0.05% Tween 20 in phosphate-buffered saline (PBS).
3. Block the plate with Reagent Diluent (0.5% BSA + 0.5% Casein in PBS, pH 7.4) 300 µl for 2 hours at room temperature.
4. Repeat the wash as in step 2. The plate is now ready for sample addition.

Assay Procedure

1. Add 100 µl of all standard serial dilutions and dialysate samples to the 96-well plate and incubate for 2 hours at 4°C.
2. Wash the plate three times with 0.05% Tween 20 in PBS.
3. Add 100 µl of polyclonal antibodies and incubate for 1 hour at room temperature.
4. Wash the plate three times with 0.05% Tween 20 in PBS.
5. Add 100 µl of conjugated antibody to each well. Cover the plate and incubate for 20 minutes at room temperature.
6. Wash the plate three times with 0.05% Tween 20 in PBS.
7. Add 100 µl of substrate solution to each well. Incubate for 20 minutes at room temperature.
8. Add 50 µl of stop solution to each well. Gently tap the plate to ensure thorough mixing.

9. The absorbances were calculated by taking measurements at 450 nm. Biomarkers concentrations were calculated based on a log-transformed standard curve.

Appendix F

Peritoneal mesothelial cell culture

- Let patients have overnight dwell of peritoneal effluent for 8-10 hours
- Drain the entire peritoneal effluent, measure the volume in mL.
- Hang the drained bag until there is sedimentation of cells
- Use sterile pipette to suck peritoneal effluent from above of the bag until there is 200 mL of the suspension.
- Transfer the suspension into 50 mL. tube (Avg. 4 tubes)
- Centrifuge the suspension at 1500 rpm with 4^{o}C for 20 minutes and then wash with PBS 2 times
- Put the remaining cells in 5-7 mL of culture medium (M199 + 20% fetal bovine serum, 100 IU/mL penicillin, 100 mcg/ml streptomycin, 2% biogro-2), count cells in counting chamber
- Incubate cells in 25-cm^2 tissue culture flask at 37 ^{o}C , 5% CO_2
- Change culture medium every 2-3 days

VITA

NAME	Panida Ditsawanon
DATE OF BIRTH	12 Nov 1985
PLACE OF BIRTH	Chiangmai
INSTITUTIONS ATTENDED	Chiangmai University