CHAPTER V
DISCUSSION

Peritoneal dialysis is a highly successful for patient survival equivalent to homodialysis during the initial 3-5 years. However, using a conventional high glucose base peritoneal dialysis fluid (GPDF) as osmotic agent induces both local and systemic effects. After 6 year therapy by this solution, major CAPD patients have peritoneum membrane alterations in the structure and function [131]. Their peritoneal membrane function with high peritoneal transport rate, loss of ultrafiltration capacity and inadequate small solute removal which is associated to high blood pressure, fluid overload, cardiovascular problem, metabolic alterations and patho-physiological changes lead the patient to the risk of mortality [9]. Therefore, type of peritoneal dialysis used and regimen strategies to manage patient to preserve the peritoneal membrane functions and to promote ultrafiltration capability are important [133].

Glucose polymer corn derivative based PDF (CPDF) represent a significant improvement for PD therapy on clinical outcome because it preserves cells viability and reduces peritoneal membrane alterations [206] and minimize the possible systemic effects—in the form of improved nutrition, decreased inflammatory stimuli, remove fluid and solute during the long dwell and helping to reduce the mortality in cardiovascular disease. Some recent studies have suggested corn derivative-based PDF improves glycemic control, hyperinsulinemia, insulin resistance, and hyperlipidemia in diabetic and non diabetic PD patients [10, 63, 184, 190, 206].

Using GPDF as osmotic agent for peritoneal dialysis had many disadvantage, and glucose polymer CPDF is relative expensive and not affordable in Thailand. Therefore, this study aims is to investigate a new possible glucose polymer tapioca derivative based as osmotic agent for development of peritoneal dialysis fluid, TPDF.

The glucose polymer based PDF with less GDP contain may preserve peritoneal membrane compared to high glucose based PDF. It may also associate with the improvement in fluid removal, and reduce hyperglycemia complication.

Lesson we have learnt from this research, to develop the alternative polyglucose based PDF, we had played an attention to the totally at least three steps (i) Preparation and characteristics of TPDF process, (ii) effects on peritoneal cells testing and acute toxicity testing in animal (iii) efficacy to induce water osmosis and its mechanism.

There are three areas involve in this study

1. Tapioca derivatives characteristics

Tapioca derivative is potential osmotic agent for development as polyglucose based PDF. Due to the molecular characteristic and structure profile of tapioca is similar to corn derivative. Both tapioca and corn derivative polymers have similar molecular weights by weight (Mw), by number (Mn) and %polydispersity or ratio of weight-average molecular weight (Mw) to number –average molecular weight. This polymer osmotic agent is stable and can induce water osmosis through a long dwell 12 hours comparable to corn derivative based PDFs and better than GCPF. It is stable and has limited glucose degradation products after stepped of heat sterilization (Table 4.1D). This indicates an advantage property of tapioca derivative based compared to glucose based and similar ranges of the examined GDPs to corn derivative based PDFs.

Our tapioca derivative had has similar property to corn derivatives (Table 4.1A) which is confirmed by both enzymatic and ^{1}NMR methods. However, it should be noted that although the same starch but from different source may have slightly different molecular characteristics contains. Different types of starches have different contains of amylose and amylopectin. Corn derivative contains more linear amylose of approximately 28%, while tapioca derivative has a lower amylose of about 17% and contains more branching amylopectin 83% (Jane, 2009). However, low molecular weight property of tapioca and cornstarch derivatives are similar with slightly higher of low MW DP2-DP6 (Table 4.1B). This result may suggest the property of tapioca derivative should be more resistible to beta amylase enzyme (an enzyme specific to cute a position of alpha 1, 4 non reducing ends).

3. The efficacy in term of safety

Toxicity of peritoneal dialysis fluids on cultured fibroblasts: The TPDF reduced the favorable effect on fibroblast cell line testing especially when compared to 4.25% GPDF. From the results it possibly suggested that decreased toxic effect of TPDF on LDH activity compared to GPDF. From this study, dilution 1 to 1 with medium for 16 hours is used as procedure of *in vivo* equilibration of the treatment. We also demonstrated with 4 hours exposure using the fibroblast cell line. It is important to remember that in the clinical situation the patients are treated for much longer periods, with fluids renewed several times daily. However, five to 60 minutes lasting incubation period of the fibroblast cell line testing in diluted and undiluted PDFs were recommended regard to pH influences or buffer system. Because, about 15 to 30 minutes equilibration of pH is reached in the peritoneal cavity of PD patients.

The present mesothelial cytoxicity testing has demonstrated that TPDF did not induce mesothelial morphologic changes, cell injuries, and apoptosis. No major difference was observed between the two glucose polymers in their effects on mesothelial viability. It appears a possibility of using TPDF also in clinical application. GPDF influence on mesothelial cells similar to previous reports [43]. The results in the present *in vito* cell cultures study have demonstrated that the effects of high dose of glucose, not either 1.5%GDPF and polygluocse, contributes significant mesothelial cell injury similar to previously reported [42, 43, 66, 67, 151, 218]. The early state of apoptosis as a form of programmed cell death was tested by examining of the translocation of phophatidylserine to the cell surface using flow cytometry with Annexin V/PI staining. However, the results did not show a significantly difference between the tested solutions. The % cell death is reduced among polyglucose based PDF, this may suggest the more its biocompatibility as similar as previous studies [42, 158].

Buffer system tested after adjusted pH, the test fluids with glucose polymer both tapioca and corn based PDFs showed a better mesothelial and fibroblast cell viability compared to high glucose of 4.25% contained PDF with having the negative impact of high GDPs contained PDF. It may suggest that TPDF is relative stable although mixing

with electrolytes and buffered. However, a high colloid osmotic pressure would be also influenced on the cell functions as the effects have been seen if testing with a higher %TPDF. This result suggests the higher doses of glucose polymer based PDF administration per day should be warranted.

PBMC were selected as one of peritoneal cells precursors of macrophages this cell plays a significant role in immune defense mechanism, whether it has been affected after being exposed to the tested peritoneal dialysis fluid. PBMC toxicity was determined as an inhibition of cell growth by quantification of cell proliferation. Polyglucose itself is effective used as peritoneal dialysis and no effect on cell proliferation compared to glucose based PDF. TPDF caused significant inhibition of the growth of PBMC. This study, we confirmed that filtered instead of autoclaved TPDF, pH and GDPs were not the causes. PBMC is immune cells responsible to antigen stimulation; the toxicity of cell proliferation reduction might be related to the endotoxin contamination. We believe, if the level of endotoxin is limited, the perseverance of cell growth will be obtained, the research is ongoing.

We preliminary examined toxicity in mice. There were no deaths during 2 weeks duration of acute toxicity testing from both intravenous dose 5 ml. Kg^{-1} of 15%TPDF and intraperitoneal dose 10 ml. Kg^{-1} of 20%TPDF compared with 0.85%normal saline solution. No significant differences of body and organs weight between among control and test group. There was no significant abnormality finding from pathology and necropsy examination.

3. The effectiveness of polyglucose derivatives to induce water osmosis and its mechanism

Glucose polymer has large molecule, thus it is difficult to transport across the peritoneal capillary walls, and more stable than that of glucose. It slowly gradually absorbed from the peritoneal cavity mainly through the peritoneal lymphatic system. It induces ultrafiltration (UF) through mainly the process of colloid osmosis. It is well recognized that 7.5%TPDF is iso-osmolar but can induce water osmosis through colloid

osmosis, while hyperosmolar 4.25%GPDF induces through crystalloid with has more rapid peritoneal absorption [61, 149].

The efficacy of tapioca derivative molecule to induce water osmosis is affirmed in our water osmosis studies in both *in vitro* and mathematics model (Fig. 4.4.1A & Fig. 4.4.1B). It can induce water osmosis comparable to corn derivatives. Cellophane bags with MWCO 3.5 kDa, have a larger reduction of mass change of about 71% without the small molecules. The smaller the effective pore size, the larger the "crystalloid" effect becomes (Fig. 4.4.2D). The physiochemical properties of glucose polymer property are dependent on their molecular distribution and oligosaccharide profiles. This study clearly shows that tapioca derivative based PDF is potential used as effective osmotic agent. Its molecular weight characteristic and properties can induce water transportation. Arising from our results, a process development program we demonstrated its capability to induce water osmosis comparable to corn based peritoneal dialysis fluid and sustain longer water osmosis than glucose based peritoneal dialysis.

The small molecule effect on water osmosis by its diffusive properties (Fig.4.4.2B) to increase reflective coefficient of the diffusible small molecules to draw back water flow from plasma side into dialysis fluid side this will increase the role of small diffusive molecule. This result confirmed that the small molecular weight fractions played more effective role when MWCO barrier (Fig. 4.4.2E). From this study, we question what specific molecular weight range molecule can induce the difference water osmosis in each stage of peritoneum changes, it needs further investigation.

In conclusion

TPDF has a neutral pH and very low glucose degradation products. *in vitro* cytotoxicity testing shows the tapioca derivative mixture has a significantly improved biocompatibility profile. Studies on peritoneal cells demonstrate improved cell viability, proliferation, decreased cytotoxicity and preserved cell growth in fibroblast cultures as well. *In vitro* biocompatibility testing has clearly demonstrated that are superior to

conventional solutions in preserving cell viability and function in peritoneal cell populations. TPDF is superior to conventional 4.25%GPDF and similar to that of the 7.5%CPDF or 1.5%GPDF. This new solution may have a favorable impact on peritoneal membrane integrity. Both TPDF and CPDF formulations are more biocompatible than a conventional 4.25%GPDF in term of pH and osmolarlity.

The result from this study indicated that a new glucose polymer based, TPDF, has equal efficacy as CPDF to induce water osmosis and better than glucose based PDF. However, different glucose polymers with different dextrose equivalent properties and molecular compositions it has different rate of enzyme digestion that may affect the solubility and bioavailability and therefore different functionality in a particular interested application that need further investigation.

This positive assessment now provides a foundation and rationale for stepping forward with the next stages in pharmacokinetics in vivo animal models and ex vivo metabolism studies.