Effects of Low-glucose Degradation Product Solution on Peritoneal Membrane Characteristics in Peritoneal Dialysis Patients

A 3-year Follow-up Study

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Introduction. Changes in peritoneal membrane characteristics including epithelial-mesenchymal transition are an important problem in the maintenance of peritoneal dialysis (PD). This study reports a 3-year follow-up assessment of the effects of low-glucose degradation products (GDP) solution, including epithelial-mesenchymal transition.

Materials and Methods. Adult patients who received continuous ambulatory PD between April 2001 and March 2007 were identified, and those who maintained on PD with the same solution for more than 3 years were included. Patients with an initial effluent score of 3 (fibroblastoid cells dominant in overnight effluent dialysate) were excluded. The patients were divided into two groups according to the dialysate: standard and low-GDP. The following were measured: cancer antigen-125, cell score, normalized protein equivalent of nitrogen appearance, dialysate-plasma creatinine and sodium ratios, and residual renal function. Cell score and peritoneal equilibration test were measured at 1, 6, 12, 18, and 24 months after the initiation of PD.

Results. Fifty patients were in the standard group and 76 in the low-GDP group. No significant difference in dialysate-plasma creatinine ratio was detected at baseline and at the end-point of follow-up between the two groups. Dialysate-plasma sodium ratio decreased significantly at the end-point of follow-up in the low-GDP group. Initial and follow-up cancer antigen-125 levels were higher in the low-GDP group. Multivariable analysis showed that low-GDP was associated with a higher cell score 3-free survival rate.

Conclusions. The present study shows that a low-GDP solution may be associated with preserving the mesothelial cell and peritoneal membrane characteristics.

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INTRODUCTION

Peritoneal dialysis (PD) is one of the most common renal replacement therapy methods for patients with end-stage renal disease (ESRD).[1,2] Change in peritoneal membrane characteristics including epithelial-mesenchymal transition (EMT) is an important problem in the maintenance of PD.[3] Previous studies showed that the glucose degradation products (GDP) (acetaldehyde, formaldehyde, methylglyoxal, 3-deoxyglucosone,

and 3,4-dideoxyglucosone-3-ene) in a glucose-based dialysate is a major factor for the development of morphologic and functional changes in the peritoneal membrane.[4] Glucose degradation products are thought to damage mesothelial cells and disturb remesothelialization. Furthermore, chronic exposure to GDP is associated with increased vascularization and fibrous peritoneum, ultimately resulting in ultrafiltration failure.[5]

The formation of GDP is reduced by utilizing a 2-compartment system, separating the high glucose component with low pH from the lactate buffer solution. The solutions in the two compartments are mixed prior to infusion; the pH of the final solution ranges between 6.8 and 7.4 and contains a low amount of GDP.[6] Many studies have shown that a low GDP solution has systemic and local benefits.[4-6] However, previous studies had a relatively short duration of follow-up or a small number of patients. Therefore, we reported on our 3-year follow-up assessment of the effects of low-GDP solution, including EMT.

MATERIALS AND METHODS

Selection of Patients

We reviewed medical records at Yeungnam University Hospital in Korea and identified adult patients (> 18 years of age) who received continuous ambulatory PD between April 2001 and March 2007. Among these incident patients, those who maintained PD with the same solution for more than 3 years were included. During the first month after the initiation of PD, the cells in the overnight effluent dialysate were isolated. The cells were cultured and scored according to a previously reported protocol as follows: 1, cobblestone-shaped human peritoneal mesothelial cells; 2, mixed; and 3, fibroblastoid cells dominant.[7] Among these patients, those with a score of 3 were excluded.

Finally, 126 patients were enrolled in the study. The patients were divided into the following 2 groups according to the dialysate: standard group (n = 50, Stay-safe®) and low-GDP group (n = 76, Balance®). The type of dialysate received by each patient was chosen at random. The glucose concentration, buffer, and pH of the dialysate administrated to the standard group were 1.5% to 4.25%, lactate (315.3 mg/dL), and 5.5, respectively, whereas those of the low-GDP group dialysate were 1.5% to 4.25%, lactate (315.3 mg/dL), and 7, respectively. The study protocol was approved by the institutional review board at Yeungnam University Hospital (YUH-12-0356-O27). Informed consent was waived by the board.

Clinical Information

The clinical and laboratory data collected 1 month after the initiation of PD included age, sex, underlying disease, body mass index, dialysis modality (automated PD), and levels of hemoglobin, albumin, and C-reactive protein (CRP). The peritoneal membrane characteristics were assessed using the peritoneal equilibration test. The dialysate and urine were collected during the 24 hours prior to peritoneal equilibration test. Cancer antigen-125 (CA-125), cell score, weekly Kt/V, normalized protein equivalent of nitrogen appearance (nPNA), and residual kidney function (RRF) were measured. For peritoneal equilibration test, the intra-abdominal fluid was drained, and PD fluid containing 4.25% glucose was infused intraperitoneally. The creatinine level of the drained dialysate 4 hours after the injection was divided by that of blood to obtain the dialysate-plasma creatinine ratio. The sodium level of the drained dialysate obtained 1 hour after injection was divided by the serum sodium obtained immediately before the injection to obtain the dialysate-plasma sodium ratio. In addition, cells in the overnight effluent dialysate were completely isolated by centrifugation. The cells were then cultured and scored using a previously published protocol.[7] Levels of CA-125 were measured in the overnight effluent dialysate using Abbott Architect i2000 (Abbott Diagnostics, Abbott Park, IL, USA). Dialysate CA-125, dialysate-plasma creatinine ratio, dialysate-plasma sodium ratio, and cell score were measured at 1, 6, 12, 18, 24, 30, and 36 months after the initiation of PD. Peritoneal dialysis-associated peritonitis was defined as a symptom or sign (abdominal pain, fever, and turbid dialysate) combined with an effluent cell count of more than 100/μL leukocytes, with at least 50% polymorphonuclear neutrophilic cells.[8] Peritonitis was indicated as episodes per year.

Statistical Methods

Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 19.0, SPSS Inc, Chicago, Ill, USA). Continuous variables (age, body mass index,

hemoglobin, CRP, nPNA, albumin, RRF, weekly Kt/V, dialysate-plasma creatinine ratio, dialysate-plasma sodium ratio, and CA-125) were expressed as mean ± standard deviation. Categorical variables (sex, underlying disease, and cell score) were expressed as counts and percentages. Differences in continuous variables were compared using the *t* test. Differences in categorical variables were compared using the Pearson chi-square test or the Fisher exact test, as appropriate. Survival rates from cell score 3 in the overnight effluent were calculated using the Kaplan-Meier and the Cox regression analyses. The statistical power using a sample size of 126 patients was 71.4%. *P* values less than .05 were considered significant.

RESULTS

Baseline Characteristics

The prevalence of DM was higher in the low-GDP group than in the standard group (Table 1). No significant differences in sex, body mass index, hemoglobin, CRP, nPNA, albumin, RRF, weekly Kt/V, and baseline cell score were detected between the two groups at the initiation of PD. During a 3-year follow-up, no significant difference was observed in peritonitis episodes per year between the two groups (0.38 ± 0.45 times in the low-GDP group versus 0.43 ± 0.44 times in the standard group; *P* = .59).

Changes in Peritoneal Membrane Characteristics

At baseline, dialysate-plasma creatinine ratio was 0.69 ± 0.09 in the low-GDP group and 0.66 ± 0.10 in the standard group (*P* = .13). At the end-point of follow-up, dialysate-plasma creatinine ratio was 0.67 ± 0.11 in the low-GDP group and 0.69 ± 0.10 in the standard group (*P* = .89). No significant difference was detected at both baseline and the end-point of follow-up between the two groups. At baseline, dialysate-plasma sodium ratio was 0.891 ± 0.027 in the low-GDP group and 0.890 ± 0.029 in the standard group (*P* = .94; Figure 1). At the end-point of follow-up, dialysate-plasma sodium ratio was 0.881 ± 0.030 in the low-GDP group and 0.887 ± 0.029 in the standard group (*P* = .27). A significant decrease

Table 1. Baseline and Follow-up Characteristics of Patients on Peritoneal Dialysis*

Parameter	Low-GDP Group (n = 76)	Standard Group (n = 50)	*P*
Baseline Measurements			
Age, y	53.8 ± 13.3	52.7 ± 13.7	.65
Male (%)	37 (48.7)	26 (52.0)	.72
Diabetes as underlying disease (%)	48 (63.2)	21 (42.0)	.02
Body mass index, kg/m^2	23.5 ± 3.2	23.3 ± 3.5	.77
Hemoglobin, g/L	102 ± 12	102 ± 16	.92
C-reactive protein, mg/L	5.3 ± 16.3	6.4 ± 19.0	.74
nPNA, g/kg/d	0.86 ± 0.23	0.91 ± 0.23	.28
Plasma albumin, g/L	34.4 ± 5.9	35.9 ± 5.5	.14
RRF, mL/min/1.73 m^2	3.87 ± 2.58	2.98 ± 2.19	.05
Weekly Kt/V	2.52 ± 0.56	2.33 ± 0.59	.09
Dialysate-plasma creatinine ratio	0.69 ± 0.09	0.66 ± 0.10	.13
Dialysate-plasma sodium ratio	0.891 ± 0.027	0.890 ± 0.029	.94
Cancer antigen 125, U/mL	45.8 ± 23.3	17.0 ± 10.2	< .001
Cell score			.10
Cobblestone-shaped HPMC (%)	59 (77.6)	32 (64.0)	
Mixed (%)	17 (22.4)	18 (36.0)	
Last Follow-up measurements			
Hemoglobin, g/L	110 ± 11	105 ± 10	.04
C-reactive protein, mg/L	5.3 ± 10.9	4.9 ± 1.2	.89
nPNA, g/kg/d	0.82 ± 0.18	0.83 ± 0.17	.76
Plasma albumin, g/L	37.0 ± 5.7	37.1 ± 5.4	.97
RRF, mL/min/1.73 m^2	2.01 ± 3.18	1.57 ± 1.82	.44
Weekly Kt/V	2.03 ± 0.49	2.06 ± 0.35	.84

*GDP indicates glucose degradation product; nPNA, normalized protein equivalent of nitrogen appearance; RRF, residual renal function; and HPMC, human peritoneal mesothelial cell.

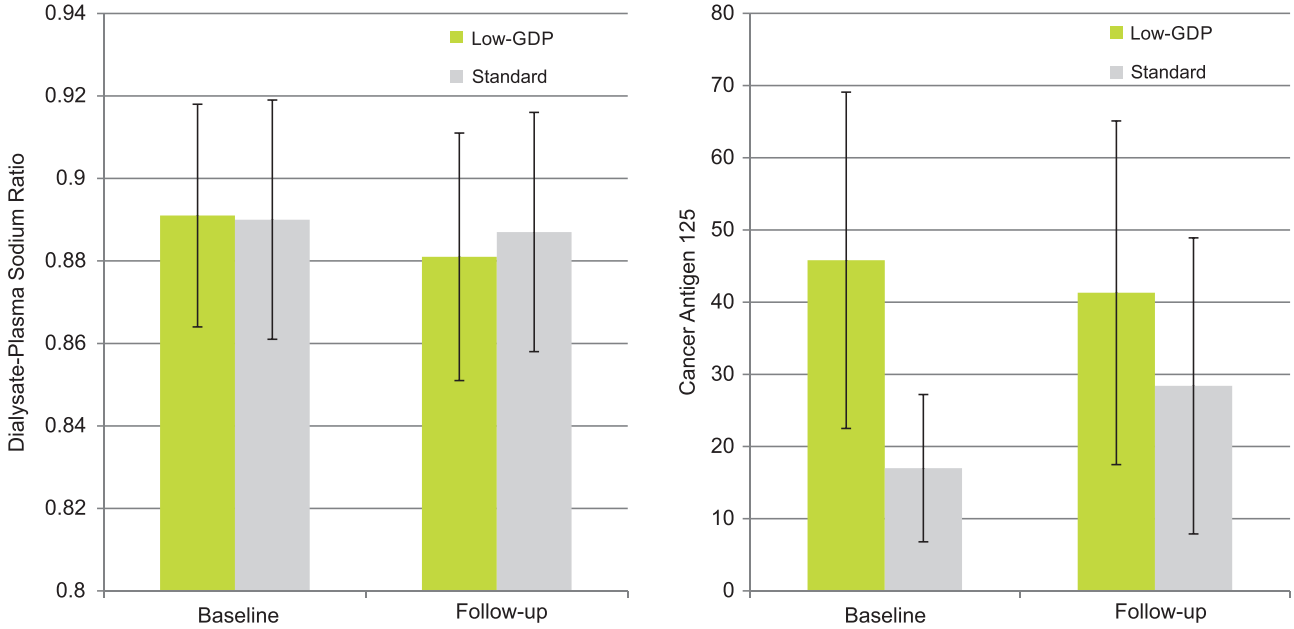

Figure 1. Changes in peritoneal membrane characteristics between the Low-glucose degradation product (GDP) group and the standard group between baseline and end-point follow-up.

in dialysate-plasma sodium ratio was detected in the low-GDP group (P = .03). At baseline, CA-125 was 45.8 ± 23.3 U/mL in the low-GDP group and 17.0 ± 10.2 U/mL in the standard group (P < .001). At the end-point of follow-up, CA-125 was 41.3 ± 23.8 U/mL in the low-GDP group and 28.4 ± 20.5 U/mL in the standard group (P = .004). The CA-125 level was higher in the low-GDP group than in the standard group. Subsequently, this trend was maintained at the end-point of follow-up.

Patient Survival

The Kaplan-Meier curve showed that the cell score 3-free survival rate in the low-GDP group was 89.2% at 1 year and 56.7% at 3 years (Figure 2). These values were 63.8% at 1 year and 23.0% at 3 years in the standard group. The low-GDP group was associated with a higher cell score 3-free survival rate (P = .001). The effects of independent variables on survival from cell score 3 are described in Table 2. In univariable and multivariable analyses, the low-GDP group was associated with a higher cell score 3-free survival rate.

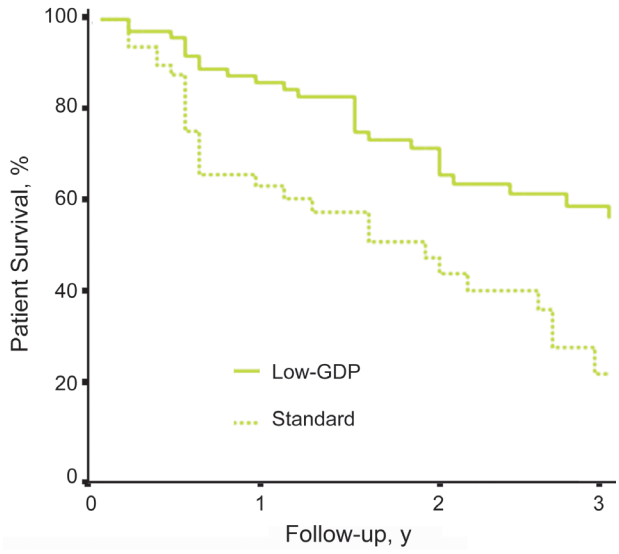

Figure 2. Kaplan-Meier score 3-free survival curve by dialysate (3-year survival rates, 56.7% for the low-glucose degradation product [GDP] group, 23.0% for the standard group; P = .001).

Table 2. Univariable and Multivariable Cox Proportional Hazard Analysis for Cell Score 3-free Survival*

	Univariable Analysis		Multivariable Analysis	
Variables	**Odd Ratio (95% CI)**	***P***	**Odd Ratio (95% CI)**	***P***
Low-GDP group	0.418 (0.244 to 0.717)	.002	0.458 (0.263 to 0.797)	.006
Diabetes mellitus	1.034 (0.606 to 1.766)	.90	to	-
Initial cell score (mixed)	1.857 (1.067 to 3.231)	.03	1.546 (0.875 to 2.731)	.13

*GDP indicates glucose degradation product and CI, confidence interval.

DISCUSSION

In the present study, we showed that the low GDP was associated with a high CA-125 level at both baseline and the follow-up. Although no significant difference in baseline dialysate-plasma sodium ratio was detected between the two groups, dialysate-plasma sodium ratio was higher in the standard group than in the low-GDP group at the end point of follow-up. In univariable and multivariable analyses, the low-GDP group was associated with a high cell score-3 free survival rate.

Water transport in PD is associated with the hydrostatic pressure gradient, the colloid and the crystalloid osmotic pressure gradient.[9] Free water transport occurs exclusively through the ultrasmall pore or the aquaporin-1 irrespective of solute transport.[9,10] The dip in dialysate-plasma ratio of sodium has been recognized as a method for measuring aquaporin. The preservation of aquaporin-1 is important to prevent ultrafiltration failure. Decreased free water transport may be associated with reduced expression of aquaporin and with functional impairment.[11-13] Two in vivo studies showed that a biocompatible dialysis solution was associated with better preservation of aquaporin.[14,15] However, few human studies have assessed the effect of a low-GDP solution on aquaporin. The Euro-Balance Trial showed that a low-GDP solution was not associated with peritoneal membrane characteristics despite the fact that clinical parameters suggested an improvement.[16] However, the Euro-Balance Trial did not evaluate aquaporin function and had the limitation of a short-term follow-up. In the present study, long-term follow-up showed no significant difference in solute transport; however, the dialysate-plasma sodium ratio in the low-GDP group was lower than that in the standard group. Further investigations are needed to evaluate whether this change is associated with reduced expression or functional impairment of aquaporin.

The CA-125 is a glycoprotein with a molecular weight exceeding 200 000 Da in gel filtration experiments.[17] It is known as a marker of peritoneal mesothelial cell mass.[18] Although few studies have examined the association between CA-125 and peritoneal parameters, CA-125 is not only a simple marker of mesothelial cell mass, but also an adjusting marker for the identification of growth factors secreted by mesothelial cells.[7] Previous studies showed that a low-GDP solution was associated with high CA-125 levels; however, most studies reporting such results were short-term studies of less than 12 to 13 months.[7,16,19] The results of the present study showed that the dialysate CA-125 was higher in the low-GDP group than in the standard group during a 3-year period.

Epithelial-mesenchymal transition is an important in the pathogenesis of peritoneal fibrosis during PD, renal fibrosis, cancer progression, and embryogenesis.[3,20,21] Immunoblotting or real-time polymerase chain reaction for the detection of EMT markers has been used to quantify EMT in in vivo and in vitro studies. However, these methods mainly require peritoneal tissue, which is difficult to obtain in clinical research. Ex vivo studies using tissues detached from the peritoneum have been performed to quantify the EMT in this field. In the present study, cell scores based on morphologic classification were used to quantify EMT. The cell score system as a prognostic factor is categorized as variable. Therefore, we enrolled patients with initial cell scores of 1 or 2 and performed survival analysis using cell score 3 as the end-point. In univariable and multivariable analyses, a low-GDP solution was associated with a protective effect on the progression from a mesothelial cell to a fibroblastoid cell, ex vivo. This statistical analysis method could be important for comparing factors affecting EMT in clinical research.

The present study showed that baseline CA-125 was higher in the low-GDP group than in the standard group. Previous studies have shown that a low-GDP solution is associated with increased effluent levels of CA-125, which is an indicator of mesothelial cell mass.[22-26] In addition, a low-GDP solution is beneficial for the preservation of RRF.[23,27] In our center, baseline CA-125 and RRF were evaluated at 1 month after the start of PD. Baseline CA-125 and RRF were higher in the low-GDP group than in the standard-group. The use of a low-GDP solution for 1 month may have affected these two variables. Kim and coworkers showed that baseline CA-125 at 1 month was higher in patients using a low-GDP solution than in those using a high-GDP solution.[28] In addition, RRF in patients using a low-GDP solution was greater than in those using a high-GDP solution, although this result was not statistically significant. The aim of the present study was to evaluate specific

effects associated with the use of a low-GDP solution, including EMT. It is difficult to evaluate the independent effect of low-GDP dialysate on RRF or the levels of CA-125. Using baseline values before 1 month may be of help to obtain similar baseline CA-125 or RRF values.

This study was a retrospective study with a small sample size and has inherent limitations such as selection bias and accidental bias. The types of dialysate used by the patients were randomly chosen. Only 126 of the total incident PD patients were included in the study. The prevalence of DM differed between the two groups. However, DM per se was not associated with cell score 3-free survival. Prospective randomized controlled studies will be needed to overcome these limitations. In addition, we were unable to evaluate the effect of PD duration. The present study enrolled only incident PD patients and it is difficult to define the effect of PD duration. Therefore, a cross-sectional study using prevalent patients may be useful.

CONCLUSIONS

In summary, this 3-year follow-up study provides evidence that the low-GDP solution is associated with a protective effect on the progression to EMT and high effluent CA-125, and may be associated with the improvement of aquaporin function. Therefore, a low-GDP solution may help improve peritoneal membrane characteristics and preserve mesothelial cells during long-term follow-up.

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CONFLICT OF INTERESTS

None declared.

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