Continuous Ambulatory Peritoneal Dialysis Improves Both The Number and Memory Function of CD4 T Cells in Uremic Patients

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Cell-mediated immune response (CMIR) was studied in 16 ESRD (end-stage renal disease) patients prior to and after 6 months of treatment with CAPD (continuous ambulatory peritoneal dialysis). Quantitative assessment of the CMI system showed that the mean values of number and percentage of total lymphocyte count, CD4, CD8, and CD4/CD8 in ESRD patients were lower than in the normal population. Such values, however, were significantly increased after 6 months of CAPD treatment. To determine qualitative function of the CMI system, both in vitro (PHA stimulation test) and in vivo (multi CMI skin test) tests were examined. There were no significant changes in the results of PHA stimulation test after 6 months of CAPD treatment. In multi CMI skin test, the number of patients converting from negative to positive result was obviously noted following CAPD therapy for 6 months. In conclusion, both quantitative and qualitative CMI impairment existing in ESRD patients could be corrected, although not completely, by 6-month CAPD treatment.

Keywords : *Cell-mediated immune response, CAPD*

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Patients with end stage renal disease (ESRD) have defects in various host defense mechanisms including phagocytosis, complements, humoral mediated immune response (HMIR), and cell-mediated immune response (CMIR)[1,2]. Clinical evidence supporting defective CMIR in ESRD is a higher incidence of malignancy than normal, cutaneous anergy, decreased immune response to vaccine, and higher carrier state after hepatitis B viral infection[1].

Quantitative as well as qualitative defect of CMIR has been demonstrated in ESRD patients[1-5]. Data regarding the effect of continuous ambulatory peritoneal dialysis (CAPD) treatment on the quantitative defect of CMIR have not been established[6-8]. Currently, there are scarce data regarding the immunologic tests of the qualitative defect in CAPD-treated patients. Indeed, on the basis of immunological test, the qualitative abnormalities of CMIR comprise three degrees of severity including abnormal response to delayed hypersensitivity skin test, impairment in mixed lymphocyte culture test, and defect in lymphocyte activation test[9].

The present study was performed to examine the status of CMIR in ESRD patients prior to and after 6 months of CAPD treatment.

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Patients and Method

Patients

The effects of treatment with CAPD on CMIR were studied in 16 ESRD patients receiving therapy at King Chulalongkorn Memorial Hospital, Bangkok Thailand. The study was approved by the Ethical Committee, Faculty of Medicine, Chulalongkorn University, Bangkok. Each patient participating in the study gave informed consent. All patients were at least 15 years old. Exclusion criteria were 1) having malignancy, cirrhosis, and active systemic lupus erythematosus and 2) receiving corticosteroid or immunosuppressive drugs.

Method

The following data were collected at the time prior to and after 6 months of treatment with CAPD

1) Nutritional assessment by anthropometry

Anthropometric nutritional parameters which were determined in the present study were body mass index (BMI) and sum of skinfold of biceps, triceps, subcapsular, and suprailiac areas[10,11]. The values of the parameters of at least 90%, 60-90%, and below 60% of normal represented normal nutrition, mild, and moderate malnutrition, respectively.

Dietary protein intake was indirectly assessed by calculating normalized protein catabolic rate (nPCR)[12].

2) Laboratory Evaluation

A) Measurement of basic laboratory

Blood samples were analyzed for hematocrit, blood urea nitrogen (BUN), creatinine (Cr), serum alkaline phosphatase, Ca^{2+}, PO_4^{3-}, cholesterol, albumin, intact parathyroid hormone (PTHi), ferritin, and serum iron.

B) Measurement of parameters associated with CAPD

Peritoneal equilibration test (PET) was performed in each patient to asses the characteristics of solute transporters[13]. To determine adequacy of dialysis, the values of weekly Kt/V and weekly normalized creatine clearance (nCCr) were measured in each patient[14].

C) Assessment of CMIR

1. Quantitative assessment

Helper T cell (CD_4) and suppressive T cell (CD_8) were determined by flow cytometry using rabbit anti-mouse immunoglobulin labelled with fluorescein isothiocyanate (FITC)[15].

2. Qualitative assessment

T cell function was assessed in both in vivo and in vitro. Regarding the former, delayed hypersensitivity skin test was determined by multi CMI skin test[16]. There were 7 antigens used in the test and these included 1) Tetanus-antigens 550,000 M rieux units/ml. 2) Diphtheria-antigens 1,110,000 M rieux units/ml. 3) Streptococcus-antigen (grpup C) 2,000 M rieux units/ml. 4) Tuberculin (old)-antigen 300,000 IU/ml. 5) Candida (albicans)-antigen 2,000 M rieux units/ml. 6) Trichophyton (mentagrophytes)-antigen 150 M rieux units/ml. and 7) Proteus (mirabilis)-antigen 150 M rieux units/ml. Glycerin solution at the concentration of 0.70 g/ml. was used as the control. The results of multi CMI skin test were assessed as "positive" when the patient positively responded to more than 4 antigens.

In vitro test of T cell function was performed by phytohemagglutinin (PHA) test[17]. Such test represents the capability of lymphocyte activation. In brief, peripheral blood mononuclear cells (PBMC) were separated from heparinized whole blood by Ficoll-Hypaque density gradient centrifugation. The PBMC were adjusted as 2×10^6 cells/ml. One hundred microlitre, of PBMC were then cultured in the presence of 100 ml of PHA, at the concentrations of 0.2 and

1mg/well, in triplicate wells including PBMC without PHA as background or negative control. The culture was maintained for 3 days (approximately 72 hours) in 37 C humidified 5% CO_2 incubator. At 6 hours prior to the end of the 3-day incubation, the culture was pulsed with 0.5 mCi/ml of ^{3}H-thymidine/well. The culture was then harvested onto a glass fiber filter to trap the DNA. Then, the filter was subjected to a liquid scintillation counter (b-counter). The resulting count per minute (cpm) was used for stimulation index (S.I.) calculation. The S.I. was the ratio of cpm obtained from mitogen-stimulated well/control well. The S.I. value of greater than 5 was considered as positive for the assay.

Statistical analysis

All data in figures and tables are expressed as means $\pm$ SD. Statistical significance was considered when $p < 0.05$. The results of all parameters prior to and 6 months after CAPD therapy were compared by unpaired T test (Table 1 and 2). Wilcoxin Signed-rank test and Mann-Whitney test were used in comparing the results of PHA stimulation test among control, prior to, and 6 months after CAPD therapy groups (Fig. 1).

Results

Of the 16 stable CAPD patients participating in the study, there were 6 males and 10 females. The age of the patients ranged from 36 to 82 years (mean $\pm$ SD = 61.5 $\pm$ 14.7 years; median = 64 years). The underlying diseases of ESRD were diabetes mellitus (n = 5), hypertension (n = 3), chronic glomerulonephritis (n = 4), chronic tubulointerstitial nephritis (n = 1), and unknown etiology (n = 3). All patients received oral supplementation of iron and folic acid. Basic laboratory data in the studied ESRD patients prior to CAPD treatment were glomerular filtration rate = 2.84 $\pm$ 0.18 ml/min, serum creatinine = 13.4 $\pm$ 0.4 mg/dl, intact parathyroid hormone = 152.9 $\pm$ 8.7 pg/ml, serum ferritin= 658.4 $\pm$ 27.3 ng/dl, serum iron = 81.0 $\pm$ 4.8 mg/L.

From the peritoneal equilibrium test, the patients were classified as: high transporter = 1 (6.25%), high average transporter = 5 (31.25%), low average transporter = 8 (50%), and low transporter = 2 (12.5%). Such transporter characteristics would indicate that CAPD was the appropriate modality of treatment for ESRD patients in the present study. The values of total weekly Kt/V, mean $\pm$ SD = 2.9 $\pm$ 3.3 and median = 2.2, and weekly nCCr, mean $\pm$ SD = 75.2 $\pm$ 2.2 and median = 73.3 L/week/1.73 m^2, all of which indicated the adequacy of dialysis.

Comparison between the biochemical parameters of ESRD patients prior to and after 6 months of CAPD treatment is displayed in Table 1. The levels of hematocrit and serum alkaline phosphatase were significantly increased ($p < 0.05$). There was a statistically significant decrease in serum phosphate concentrations ($p < 0.01$), whereas, no alteration in serum calcium levels was detected.

Regarding nutritional assessment, the values of BMI, mean $\pm$ SD = 21.1 $\pm$ 2.7 and median = 20.8 kg/m^2, represented early malnutrition. Prior to CAPD treatment, the patients, however, had values of subcutaneous skinfold thickness of less than 60% of the normal control (19.3 $\pm$ 7.9 vs 38.0 $\pm$ 7.1 mm, $p < 0.01$), indicating the status of severe malnutritional status. As shown in Table I, such values were significantly increased after 6 months-duration of CAPD treatment (25.3 $\pm$ 10.3 mm, $p < 0.05$). The concentrations of serum albumin and cholesterol were unaltered after CAPD therapy (Table 1).

Prior to CAPD treatment, the number of total white blood cells was in normal range and was not changed after 6 months of therapy (Table 2). The

Table 1. Comparison between biochemical and nutritional parameters of ESRD patients prior to and after 6 months of CAPD treatment

Parameters	CAPD Treatment		P-value
	Prior to	6 months after	
Biochemical parameters			
Hematocrit (%)	25 1.29(26)	30 1.81(32)	$p < 0.05$
Serum Ca^{2} (mg/dl)	9.1 0.31(9)	9.0 0.21(9)	NS
Serum PO_4^{3-} (mg/dl)	5.6 0.49(6)	4 0.44(4)	$p < 0.01$
Serum alkaline phosphatase (unit/L)	111.7 21.37(107)	153.7 26.41(132)	$p < 0.01$
Nutritional parameters			
Subcutaneous skinfold (mm)	19.3 2.04(19.3)	25.3 10.3(25.3)	< 0.05
Serum albumin (gm/dl)	3.49 0.47(3.4)	3.72 0.78(3.8)	NS
Serum cholesterol(mg/dl)	217.8 44.3(222)	215.3 94.0(207)	NS

Data were expressed as mean SD and median (in parenthesis).
n = 16 in each group
* $p < 0.05$; ** $p < 0.01$; NS = non significant

Table 2. Comparison between CMIR in ESRD patients prior to and 6 months after CAPD treatment

Parameters	CAPD Treatment		P-value
	Prior to	6 months after	
White blood cell (WBC) (cell/cu mm^3)	6841 1593(6755)	7271 1377(6900)	NS
Lymphocyte			
• number (cell/cu mm^3)	1338 505(1268)	1935 611(1709)	< 0.01
• percentage (when compared with total WBC)	20.2 8.6(17.3)	27.4 9.8(24.3)	< 0.01
CD_4			
• number(cell/cu mm^3)	456 220(415)	726 287(606)	< 0.005
• percentage (when compared with total WBC)	33.9 6.8(34.5)	37.8 8.5(38)	< 0.01
CD_8			
• number (cell/cu mm^3)	392 204	444 202	< 0.01
• percentage (when compared with total WBC)	29	24	< 0.05
$CD_4 : CD_8$	1.3 0.5(1.2)	1.6 0.6(1.6)	< 0.01

Data are expressed as mean SD and median (in parenthesis).
n = 16
NS = non significant

normal control values of total lymphocyte, CD_4 cells, CD_8 cells, and CD_4/CD_8 ratio were above 1500 cell/mm^3, 600 cells/mm^3 (cell count), 400 cells/mm^3 (31 $\pm$ 5% of total white blood cell count), and 1.6 $\pm$ 0.3 respectively. As obviously illustrated in Table 2, ESRD patients had lower numbers of total lymphocytes, CD_4 cells, CD_8 cells, and CD_4/CD_8 ratio than normal. Following 6 months of CAPD treatment, total white blood cell count was not altered but there were significantly increased numbers of lymphocytes ($p < 0.01$), CD_4 ($p < 0.005$), CD_8 ($p < 0.01$), and CD_4/CD_8 ratio ($p < 0.01$).

The results of multi CMI skin test prior to CAPD treatment showed that 14 ESRD patients had negative results, whereas, a positive outcome was noted in the remaining 2 patients (12.5%) (details of skin test not shown). Following CAPD treatment, there were positive results to the test in 13 patients (86.7%, $p < 0.05$, Mc Nemar test) while two patients expressed negative response. The test was not performed in one patient who died from acute myocardial infarction. Of the two negative response patients, the CD_4/CD_8 ratios were persistently lower than normal. One patient had anemia while the other was in an inadequate dialysis state.

Fig. 1 demonstrates the results of PHA stimulation test. When compared with the control, PHA at concentrations of 0.2 as well as 1 mg/well caused no significant changes in the values of stimulation index (SI) in ESRD patients before as well as after CAPD treatment.

During the study, two patients developed peritonitis from Staphylococcus aureus while exit site infection of the CAPD's catheter occurred in another two patients. All patients responded very well to antibiotics. No patients had infections from CMIR associated infection including virus, parasite, fungus, and tuberculosis.

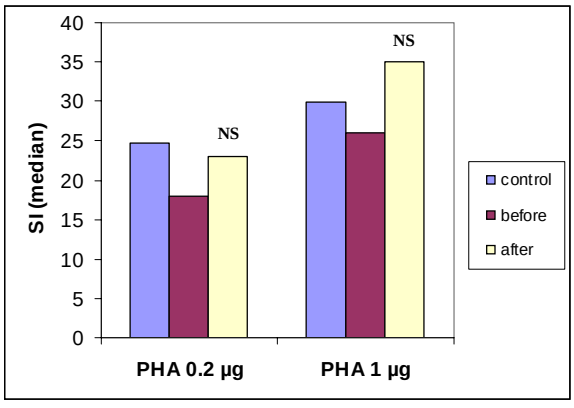

Fig. 1 Results of PHA stimulation test in ESRD patients prior to and at 6 months after CAPD treatment
Data were expressed as median
N = 15 in each group
NS = non significant when compared with control and the other group

Discussion

The results in the present study have shown that 1) ESRD patients had both quantitative and qualitative defects in CMIR. The former included a lower number of total lymphocytes, CD_4 cells, CD_8 cells, and CD_4/CD_8 ratio than normal. The latter comprised impaired multi CMI skin test. 2) Following CAPD therapy for 6 months, there was great improvement in all above quantitative as well as qualitative CMIR disorders.

That the 6 month duration of CAPD therapy in the current work could increase the number of total lymphocytes, CD_4 cells, CD_8 cells, and CD_4/CD_8 ratio is in agreement with some previous studies[6,7]. One earlier work, however, could not show such salutary effects of CAPD[8]. The discrepancy of the results among these studies might be due to the differences in adequacy of dialysis which was much lower in the past than in the present, leading to less ability to adequately eliminate uremic toxin that could impair CMIR.

In support of such a view, previous works have shown that decreased lymphocyte numbers in ESRD patients resulted from reduced thymus gland size, absence of secondary follicle in lymph node, and shortened survival of lymphocyte[1,2,18]. The important underlying factors causing all the above findings comprise uremic toxin, metabolic alterations, and malnutrition status. Middle molecular weight toxins including parathyroid hormone and guanidine derivatives have been shown to be the uremic toxins that play an important role in CMIR defect in ESRD patients. Of interest, CAPD treatment could eliminate more middle molecular weight toxins. Thus, CAPD would correct CMIR defects better than hemodialysis[19]. In this regard, earlier work has shown that hemodialysis could not effectively increase the reduced CD_4 number in ESRD[20].

Besides quantitative defect ESRD patients also have functional impairment in CMIR[1,2,21]. In the present study, most ESRD patients showed negative responses, 14 from 16 patients, to delayed hypersensitivity (multi CMI) skin test which contained 7 common antigens. This might be due to no previous exposure to that particular antigen or mediated by the

defect in CMIR. Of interest, the responses were profoundly improved, 13 from 16 patients, following 6 months duration of CAPD treatment. The response to delayed hypersensitivity skin test needs a specific recall T cell. Defective response to the test represents the earliest impairment of CMIR system[16,17]. Regarding lymphocyte activation test, when compared with control, ESRD patients showed no statistically significant difference, when compared with the control, in response to both low and high concentrations of PHA (Fig. 1). Taken together, the ESRD patients in the present study had a mild degree of functional defect in CMIR and this was improved after treatment with CAPD of 6 months.

The underlying mechanisms of such CMIR defects remain unestablished. In hemodialysis patients, it is postulated that the defects might occur at the intrinsic level of T cell or might be caused by the inhibitory effects of middle molecular weight toxins, including parathyroid hormone and guanidine derivatives, on T cell[22].

In conclusion, quantitative as well as qualitative defects in CMIR occur in ESRD patients. CAPD treatment for 6 months could effectively, although not completely, correct both abnormalities.

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การล้างไตทางช่องท้องแบบถาวรเพิ่มปริมาณ และคุณภาพของ T cell CD_4 ในผู้ป่วยไตวายเรื้อรัง

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ได้ทำการศึกษาการตอบสนองของระบบภูมิคุ้มกันชนิดอาศัยเซลล์ก่อน และหลังการรักษาด้วยการล้างไตผ่านทางหน้าท้องนาน 6 เดือนในผู้ป่วยไตวายเรื้อรังระยะสุดท้ายจำนวน 16 ราย พบว่าปริมาณ และจำนวนร้อยละของลิมโฟซัยท์ทั้งหมด ลิมโฟซัยท์ชนิด CD_4 ชนิด CD_8 อัตราส่วนระหว่างลิมโฟซัยท์ชนิด CD_4 และ CD_8 ในผู้ป่วยก่อนการล้างไตมีค่าต่ำกว่าปกติ ค่าต่าง ๆ เหล่านี้สูงขึ้นอย่างมีนัยสำคัญภายหลังการล้างไต ทำการประเมินลักษณะทางคุณภาพของระบบภูมิคุ้มกันชนิดอาศัยเซลล์โดยวิธี PHA stimulation test และ multi CMI skin test ไม่พบว่ามีการเปลี่ยนแปลงของผลการทดสอบ PHA stimulation test ในผู้ป่วยภายหลังการล้างไต แต่พบว่าจำนวนผู้ป่วยที่ให้ผลการทดสอบบวกใน multi CMI skin test เพิ่มขึ้นอย่างมีนัยสำคัญ กล่าวโดยสรุป การล้างไตผ่านทางหน้าท้องนาน 6 เดือน สามารถแก้ไขความผิดปกติทางปริมาณ และคุณภาพของระบบภูมิคุ้มกันชนิดอาศัยเซลล์ในผู้ป่วยไตวายเรื้อรัง