

Microbicidal Efficacy of Povidone-Iodine in a Noncontact Manner Applied to a Continuous Ambulatory Peritoneal Dialysis Connection System

Tetsuya Kashiwagi¹, Yasuhiko Iino¹, Hideyasu Miyahara² and Yasuo Katayama¹

¹Department of Neurological, Nephrological and Rheumatological Science, Graduate School of Medicine, Nippon Medical School

²Research and Development, JMS Co., Ltd.

Abstract

In peritoneal dialysis (PD) the dialysate is introduced into the peritoneal cavity, and the peritoneal membrane is used as the dialysis membrane. In PD, patients exchange the dialysate by themselves through the connection tube attached to the indwelling catheter that is inserted into the peritoneal cavity. Microbes may enter the peritoneal cavity during dialysate exchange, and, therefore, peritonitis is a potential complication of PD. To prevent microbial contamination, the connection tube tip is generally sealed with a protection cap containing povidone-iodine (PVP-I) during the dwelling time. This cap is designed to make direct contact with the tube tip so that microbes attached during dialysate exchange are killed by the next dialysate exchange. However, if excess PVP-I flows into the peritoneal cavity and is absorbed into the body, the complications, including thyroid dysfunction, peritoneal inflammation, and fibrous thickening, can develop.

Therefore, in this study, a new manual connection system (Zero System, JMS Co., Ltd., Hiroshima, Japan) for continuous ambulatory peritoneal dialysis was investigated to confirm that the PVP-I solution within the protection cap of the new system would not flow into the fluid passing through the tube. An experiment was also performed to confirm that the microbes on the connector tip become completely nonviable after attachment of the cap for 3 hours. The cap is fitted with a sponge containing a 10% PVP-I solution, the same as for the conventional cap system. However, the system is designed to achieve disinfection without contact, unlike with the conventional system, in which disinfection is achieved by direct contact of the PVP-I-containing sponge with the open end of the attached connector. The test results demonstrated that adequate disinfection with this system can be achieved by the next exchange, while avoiding entry of PVP-I into the peritoneal cavity from the cap.

The results suggest that the use of this connection system can avoid adverse reactions arising from the absorption of PVP-I and prevent the onset of peritonitis caused by microbial invasion of the peritoneal cavity.

(J Nippon Med Sch 2010; 77: 86–92)

Key words: povidone-iodine solution, continuous ambulatory peritoneal dialysis, new manual connection system, microbicidal efficacy of a noncontact protection cap

Introduction

In peritoneal dialysis (PD) the dialysate is introduced into the peritoneal cavity, and the peritoneal membrane is used as the dialysis membrane. At the end of 2008, 9,157 patients were receiving PD in Japan¹. Because the dialysate often contains glucose, if microbes enter the peritoneal cavity, they can propagate easily and cause peritonitis. In continuous ambulatory peritoneal dialysis (CAPD), for which the general dialysate dwelling time is 4 to 6 hours, the frequency of dialysate exchange is higher (4 times a day) than for other types of PD, and the risk of contamination is higher. The dialysate exchange is performed by the patients themselves through the connection tube attached to the indwelling catheter that is inserted into the peritoneal cavity. Therefore, contamination by the patient touching the connector connected to the dialysate bag directly affects the risk of peritonitis.

With system modifications, progress in patient education, and the increased skill of hospital staff over the past 2 decades, the frequency of peritonitis caused by contamination during dialysate exchange has decreased by at least two-thirds; therefore, CAPD has become a more reliable therapy^{2,3}. However, peritonitis due to touch contamination at the time of dialysate exchange remains a significant risk in CAPD. In fact, peritonitis is the most frequent cause of PD discontinuation (approximately 30%) in Japan, followed by death (17%), ultrafiltration failure (15%), and inadequate dialysis (11.7%)¹.

In currently available connecting systems for CAPD, disinfection is achieved with a mechanical system involving the exposure to heat or ultraviolet radiation for aseptic connection or with a manual system in which a protection cap fitted with a sponge immersed in a povidone-iodine (PVP-I) solution is attached to the connector, allowing constant contact between the connector and the solution during dialysate accumulation in the peritoneal cavity. If PVP-I flows into the body, thyroid dysfunction can occur, especially in neonates and patients with burns⁴⁻⁷; there is also a risk of peritoneal inflammation and fibrous thickening, which can trigger encapsulating peritoneal sclerosis, the most serious complication in patients undergoing

CAPD⁸. These complications can also cause ultrafiltration failure and inadequate dialysis and result in PD discontinuation. Therefore, to ensure the safety of CAPD, a system that can prevent flow of PVP-I into the peritoneal cavity is needed. The necessity of such products has been supported by cases in which hypothyroidism was induced by the PVP-I contained in the sponge fitted in the protection cap⁹.

The present study was performed to examine whether the flow of PVP-I into the peritoneal cavity can be prevented with the use of a new manual connection system (Zero System, JMS Co., Ltd., Hiroshima, Japan) designed to avoid direct contact of the connector with the PVP-I-containing sponge. An additional aim of this study was to examine whether microbes placed on the connector tip are inactivated during the 3-hour period (until the next exchange of dialysate) by the use of the new protection (Cap Kit S, JMS Co., Ltd., Hiroshima, Japan) in an *in vitro* simulation study.

Materials and Methods

PVP-I Contamination Test

A new manual connection system composed of a CAPD connection-tube connector, a dialysate-side connector, and a protection cap was used in the test group (**Fig. 1A**), and the conventional connection system manufactured by the same company was used in the control group (**Fig. 1B**). The cap in each group was fitted with a built-in sponge containing a 10% PVP-I solution. When joined to the connection-tube connector, the sponge in the cap of the new system did not come in contact with the connector, whereas in the conventional system, the sponge in the cap comes in contact with the connector.

After both caps were applied to their respective connectors, both systems were left standing for 4 hours. Then, the caps were removed, and the respective dialysate-side connectors were joined to the system, followed by the passage of 20 mL of ultrapure water through the tube. The passage through the tube of each sample of ultrapure water was investigated to quantify the effective iodine content by redox titration¹⁰, with 0.002 N sodium thiosulfate used as a reducing agent for iodine, to evaluate PVP-I contamination (n=5; **Fig. 2**).

As reference information, the amount of PVP-I

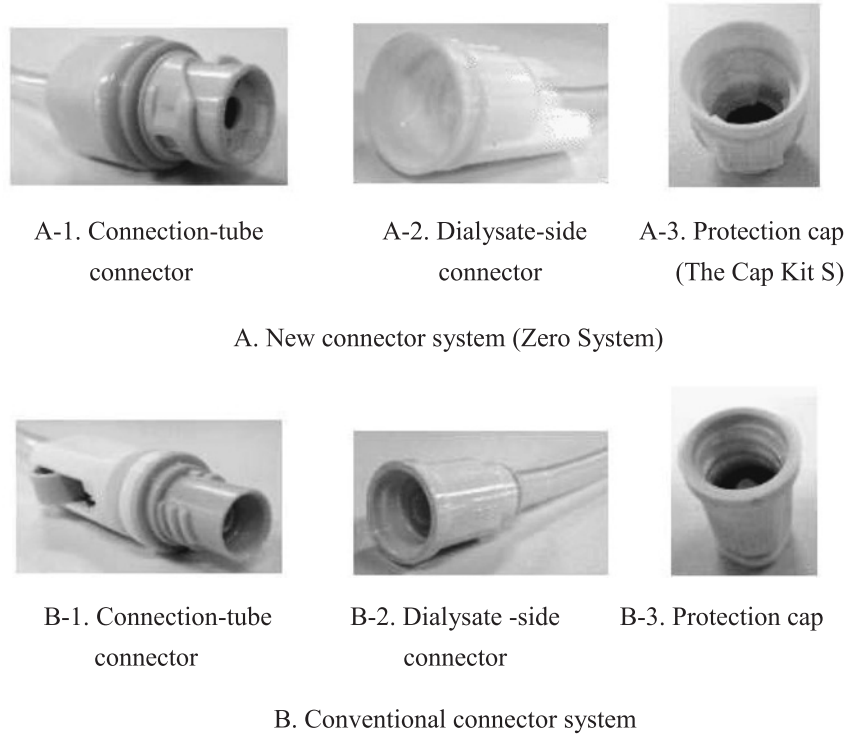
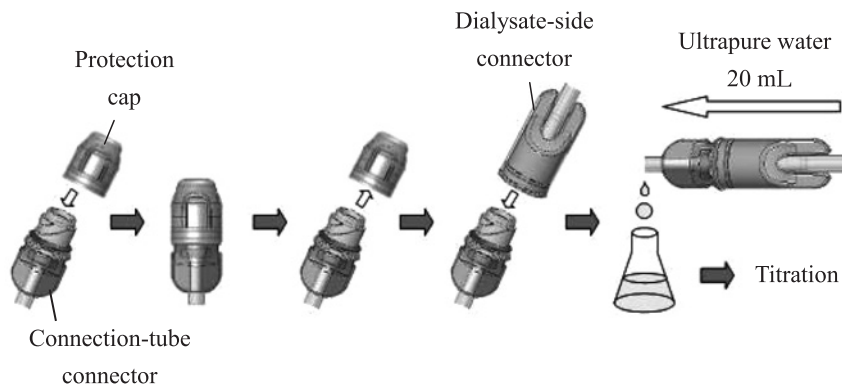


Fig. 1 Connector systems tested



1. Apply protection cap
2. Keep standing (for 4 hours)
3. Remove protection cap
4. Apply dialysate-side connector
5. Correct ultrapure water passing through tube
6. Quantify effective iodine level using redox titration

Fig. 2 PVP-I contamination test

contained in the sponge was measured by quantification of the effective iodine content in the cap before passage of the ultrapure water (n=5).

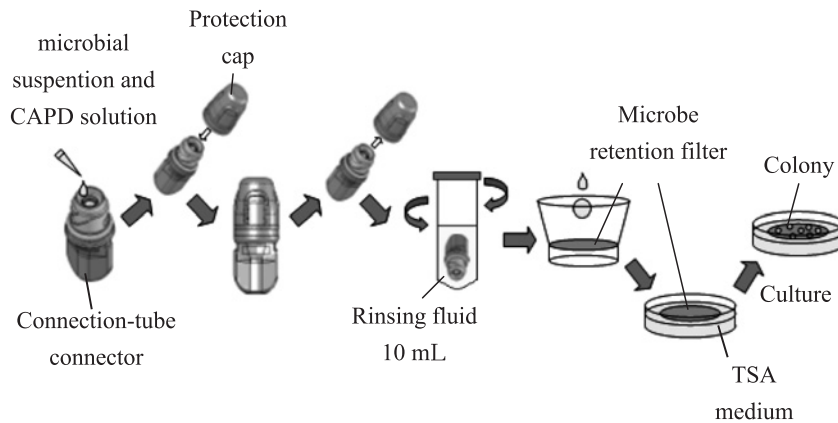
Then, the systems were compared by means of the Dunnett method for parametric variables (EXSAS ver. 4.00, Arm Systex Co., Ltd., Osaka, Japan).

The mean values and standard deviations of the quantitative results of the 5 samples were calculated.

Disinfection Test

For the test group, a new manual connection system composed of the CAPD connection-tube

Examination of a New CAPD Protection Cap



1. Place microbial suspension (10 μ L, containing 10^3 CFU) and CAPD solution (10 μ L)
2. Apply protection cap
3. Keep standing (for 3 hours at 30°C to 35°C or 10°C)
4. Remove protection cap
5. Immerse connection-tube connector in rinsing fluid, then vortex
6. Filtrate of rinsing fluid
7. Incubate filter on TSA medium

Fig. 3 Disinfection test

connector and the protection cap was used. Microbes often detected as the causative pathogens of peritonitis in patients receiving PD¹¹ were selected for this test: 2 Gram-positive bacteria (*Staphylococcus aureus* [NBRC 13276] and *Staphylococcus epidermidis* [NBRC10091]), 2 Gram-negative bacteria (*Escherichia coli* [NBRC3806] and *Pseudomonas aeruginosa* [NBRC 13275]), and 1 fungus that is often known to cause intractable infection (*Candida albicans* [NBRC1594]). The microbes were suspended (10^5 CFU/mL) in 0.01% Tween 80-supplemented physiological saline (Gram-positive bacteria) or plain physiological saline (Gram-negative bacteria and the fungus).

Each microbial suspension (10 μ L, containing 10^3 CFU) was mixed with the CAPD solution (10 μ L, Perisate 360N, JMS Co., Ltd.) and placed on the connector tip. The cap was placed over the connector tip, which was then left standing for 3 hours at 30°C to 35°C or 10°C (n=5; to achieve severe conditions the duration of cap application was shorter than the usual cap application time for CAPD of 4–6 hours). In the control group, the sponge of the cap was preimmersed in physiological saline instead of in PVP-I solution (n=1) and tested in the same way as the cap with PVP-I.

After being left standing, each cap of the test group was removed from the connector and the

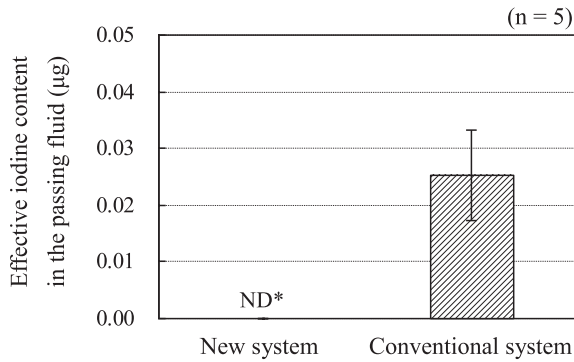
connector was immersed in 10 mL rinsing fluid (Fluid D-ST, bioMérieux, Marcy l'Etoile, France), followed by vortexing with a laboratory mixer (for 30 seconds), and subsequent collection through a 0.45- μ m-diameter cellulose membrane filter (100 mL Milliflex Filter Funnel Unit, Millipore, Bedford, MA, USA). After passage of the rinsing fluid the filter was incubated on Tryptic soy agar (TSA) medium (Milliflex Cassette Tryptic Soy Agar, Millipore) at 30°C to 35°C for 7 days, after which the colonies grown on the filter were counted. In the control group, it was anticipated that counting 10^3 CFU of microbes on a filter after the culture would be impossible. Therefore, the connector was immersed in 10 mL of rinsing solution after the standing period and subjected to vortexing with a laboratory mixer (for 30 seconds). Then, 500 μ L of the fluid was smeared onto 3 plates of TSA medium (bioMérieux) and incubated at 30°C to 35°C, followed by colony counting and calculation of the microbial count by multiplying the mean colony number on the 3 plates of TSA medium by 20 (Fig. 3).

For a quantitative evaluation of which microbial counts dispensed into the connector was 10^3 CFU/10 μ L, CFUs were enumerated by plating 100 μ L of the serially diluted solution onto TSA medium and incubating at 30°C to 35°C for 1 day for *S. aureus*, *E.*

Table 1 Results of disinfection test

							(CFU)					
Temperature		30°C to 35°C					10°C					
Test No.		1	2	3	4	5	1	2	3	4	5	
Microbial species	<i>S. aureus</i>	Sample	0	0	0	0	0	0	0	0	0	0
		Control	2.7×10^3					2.5×10^3				
	<i>S. epidermidis</i>	Sample	0	0	0	0	0	0	0	0	0	0
		Control	1.1×10^3					3.1×10^3				
	<i>E. coli</i>	Sample	0	0	0	0	0	0	0	0	0	0
		Control	4.2×10^3					3.3×10^3				
	<i>P. aeruginosa</i>	Sample	0	0	0	0	0	0	0	0	0	0
		Control	1.5×10^3					2.2×10^3				
	<i>C. albicans</i>	Sample	0	0	0	0	0	0	0	0	0	0
		Control	5.1×10^3					5.3×10^3				

*Microbial suspension placed on connector: $2.3 - 5.4 \times 10^3$ CFU



* ND: Not detectable

Fig. 4 Measurements of effective iodine content in passing fluid

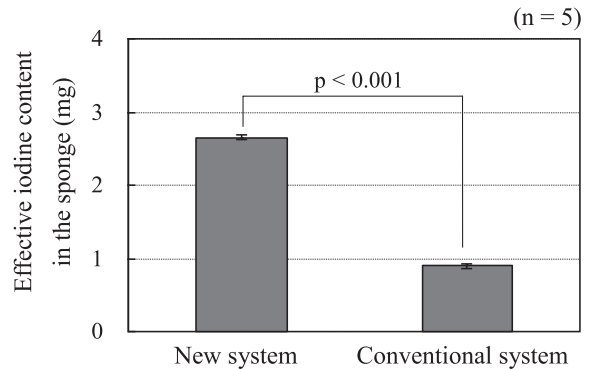


Fig. 5 Measurements of effective iodine content in sponge

coli, *P. aeruginosa*, and for 2 days for *S. epidermidis*, *C. albicans* before colonies were counted. Then, the colony counts were multiplied by the dilution ratio to estimate the total microbial load.

Results

PVP-I Contamination Test

The effective iodine content contaminating the passing fluid was $0.025 \pm 0.008 \mu\text{g}$ with the conventional cap but was less than the detection limit with the new cap (Fig. 4). The effective iodine content detected in the PVP-I solution contained in the cap sponge was about 3 times greater with the new cap ($2.65 \pm 0.04 \text{ mg}$) than with the conventional cap ($0.89 \pm 0.03 \text{ mg}$; Fig. 5).

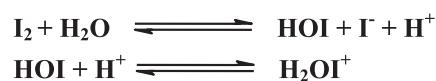
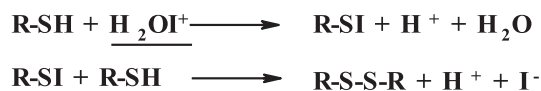
Disinfection Test

In the control group, in which the sponge was preimmersed in physiological saline instead of a PVP-I solution, all of the test microbes grew to a density of about 10^3 CFU in an atmosphere of 30°C to 35°C and 10°C. In the test group in which the new protection cap was used, all the test microbes lost viability within 3 hours in an atmosphere of 30°C to 35°C and 10°C (Table 1).

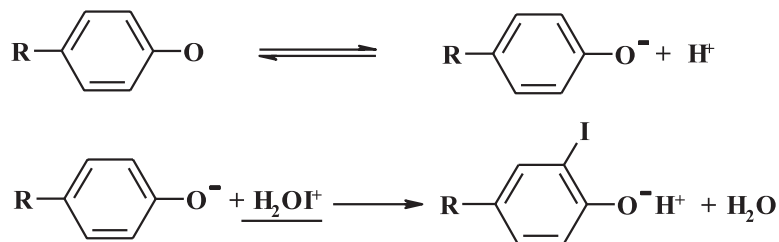
In addition, the microbial counts placed on the connector were between 2.3×10^3 to 5.4×10^3 CFU for each species.

Discussion

The present study, using a new manual connection system designed to avoid contact

1. Generation of H_2OI^+ 2. Reaction with $-\text{SH}$ Group

3. Reaction with Tyrosine



4. Reaction with Histidine

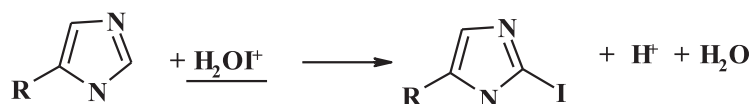


Fig. 6 Presumed disinfection mechanism of PVP-I

between the connector and the PVP-I-containing sponge, investigated whether a PVP-I solution contained in the sponge of the new protection cap contaminates the fluid passing through the tube and whether microbes adhering to the connector are inactivated (assuming touch contamination of the connector).

In this study, the results of the PVP-I contamination test confirmed the absence of contamination of the passing fluid with the new protection cap, despite the content of iodine contained in it being approximately 3-fold higher than in the conventional protection cap, in which PVP-I contamination of the passing fluid was noted. In the disinfection test, the new system was found to be capable of inactivating 10^3 CFU microbes, a much higher density than that seen following connector-touch contamination within 3 hours, which is a shorter period than that needed for dialysate exchange. Generally, less than 10^3 CFU microbes are on a human palm. With the assumption that all microbes on a palm become attached to the connector tip, 10^3 CFU was chosen as the number of microbes for this study¹².

Excess iodine intake can cause adverse effects on

thyroid function such as hypothyroidism and goiter^{13,14}. Furthermore, iodine excretion abnormality develops because of renal dysfunction in patients with end stage renal failure. Although patients treated with PD are usually euthyroid^{15,16}, elevated serum iodine levels are found in about 90% of patients receiving CAPD¹⁷. Takeda, *et al.* have reported that 3 of 93 patients receiving dialysis who had no history of thyroid diseases were found to have hypothyroidism after dialysis was started¹⁸. Because hypothyroidism in these 3 patients could be reversed by restriction of iodine-rich foods, an iodine organification defect might develop in patients receiving dialysis long term¹⁸. Moreover, in another study of the same group, PVP-I was found to have leaked from a protection cap and flowed into the peritoneal cavity to increase serum levels of inorganic iodine and thyroid stimulating hormone. Hence, entry of iodine into the peritoneal cavity is considered unfavorable even if its concentration is low.

The new manual connection system evaluated in the present study did not show contamination of the passing fluid, because contact of the connector with the protection cap sponge containing the PVP-I

solution is avoided. Furthermore, the new system could inactivate 10^3 CFU microbes within 3 hours.

According to the prevailing view of the mechanism underlying the disinfecting activity of PVP-I, the iodine (I_2) freed from the solution oxidizes water to yield H_2OI^{+19} , which then reacts with membranous protein (-SH group, tyrosine, histidine) on the microbial surface, leading to the loss of microbial viability⁷ (**Fig. 6**).

Disinfection in this contact-free new system occurs via free iodine undergoing sublimation and oxidizing the water in the air or microbes, leading to the loss of microbial viability, or via exposure of microbes to the steam of oxidized water, resulting in the loss of viability.

Conclusion

The results of the test confirmed that contamination of the passing fluid with PVP-I was avoided with the use of the new manual connection system, because the system is designed to allow no contact between the PVP-I solution (contained in the sponge of the new protection cap of Cap Kit S) and the connector. The results also confirmed that this system can inactivate 10^3 CFU microbes adhering to the connector within 3 hours of application of the new protection cap. These results suggest that various risks arising from contamination of the peritoneal cavity with PVP-I can be avoided with the use of the new system in patients undergoing CAPD, most significantly that of peritonitis caused by connector-touch contamination at the time of dialysate exchange.

References

1. Nakai S, Masakane I, Shigematsu T, et al: An overview of dialysis treatment in Japan. *J Jpn Soc Dial Ther* 2009; 42: 1–26. [in Japanese]
2. Kurihara S, Akiba T, Marumo F: Evaluation of the new sterile connecting device (TSCD SC-102) for CAPD. *Kidney and Dial* 2000; 49: 295–299. [in Japanese]
3. Imada A: Twenty year locus and latest information of CAPD relation peritonitis and exit-site infection. *Kidney and Dial* 2006; 60 (Suppl): 94–97. [in Japanese]
4. Cosman BC, Schullinger JN, Bell JJ, Regan JA: Hypothyroidism caused by topical povidone-iodine in a newborn with omphalocele. *J Pediatr Surg* 1988; 23: 356–358.
5. Vorherr H, Vorherr UF, Mehta P, Ulrich JA, Messer RH: Vaginal absorption of povidone-iodine. *JAMA* 1980; 244: 2628–2629.
6. Pietsch J, Meakins JL: Complications of povidone-iodine absorption in topically treated burn patients. *LANCET* 1976; 1: 280–282.
7. Interview form on pharmaceutical products-4th revised edition, 2005; Meiji Seika, Tokyo. [in Japanese]
8. Oseto S, Ito T, Suzuki A, Imai E: Increase tissue transglutaminase in experimental encapsulating peritoneal sclerosis. *Kidney and Dial Extra Issue* 2004; *Peritoneal Dialysis*: 273–276. [in Japanese]
9. Takeda S, Michigishi T, Ieki Y, Takazakura E: Iodine-induced hypothyroidism in a patient on nightly peritoneal dialysis. *J Jpn Soc Dial Ther* 1996; 29: 1555–1560. [in Japanese]
10. Japanese Pharmacopoeia-15th edition, 2006; pp 225–232, Ministry of Health Labour and Welfare, Tokyo. [in Japanese]
11. Imada A, Kawaguchi Y, Kumano K, Namoto Y: A multicenter study of CAPD-related peritonitis in Japan. *J Jpn Soc Dial Ther* 2001; 34: 1157–1162. [in Japanese]
12. Tomas EM, Glennie F: Touch contamination of connection devices in peritoneal dialysis—a quantitative microbiologic analysis. *Perit Dial Int* 1997; 17: 560–567.
13. Nora NA, Mujais SK: Uremic goiter: the malevolent iodine. *Int J Art Org* 1991; 14: 622–624.
14. Gavin LA, Eitan NF, Cavalier RR, Schmidt WR: Hypothyroidism induced by continuous ambulatory peritoneal dialysis. *West J Med* 1983; 138: 562–565.
15. Gardner DF, Mars DR, Thomas RG, Bumrungsup C, Misbin RI: Iodine retention and thyroid dysfunction in patients on hemodialysis and continuous ambulatory peritoneal dialysis. *Am J Kidney Dis* 1986; 7: 471–476.
16. Spector DA, Davis PJ, Helderman JH, Bell B, Utiger RD: Thyroid function and metabolic state in chronic renal failure. *Ann Intern Med* 1976; 85: 724–730.
17. Thyssen B, Gatz M, Freeman R, Alpert BE, Charytan C: Serum thyroid hormone levels in patients on continuous ambulatory peritoneal dialysis and regular hemodialysis. *Nephron* 1983; 33: 49–52.
18. Takeda S, Michigishi T, Takazakura E: Iodine-induced hypothyroidism in patients on regular dialysis treatment. *Nephron* 1993; 65: 51–55.
19. Gottardi W: Iodine and disinfection: Theoretical study on mode of action, efficiency, stability, and analytical aspects in the aqueous system. *Arch Pharm* 1999; 332: 151–157.

(Received, June 17, 2009)

(Accepted, October 13, 2009)