Journal of Advanced Engineering Vol. 2, No. 4, pp. 279-282 / October 2007

綠茶萃取物降低腎衰竭病人因血液透析所引發之動脈硬化發生率

Green Tea Extract Decreases the Risk of Atherosclerosis Induced by Hemodialysis in the Uremic Patients

陳冬生 [1] 劉紹毅 [2] 李建霖 [1] 張炎林 [*1]
Tung-Sheng Chen[1], Shaw-Yih Liou[2], Chien-Lin Lee[1], Yen-Lin Chang[*1]

摘要

末期腎衰竭病人由於長期被施以血液透析方式治療，引發血液不相容性導致併發動脈硬化機率上升。而本實驗最主要的目的，是讓腎衰竭病人服用綠茶萃取物後，觀察腎衰竭病人在血液透析前後血中動脈硬化因子如氧化壓力指標(dityrosine)、總抗氧化能力(total antioxidant status, TAS)、發炎反應指標(C-reactive protein, CRP)及凝血活化因子(tissue factor pathway inhibitor, TFPI)等濃度變化量。實驗結果顯示服用綠茶萃取物之腎衰竭病人上述四項動脈硬化指標均比未服用之腎衰竭組更接近正常值($p<0.05$)。所以本實驗建議腎衰竭病人可酌量服用綠茶萃取物來降低因血液不相容性所引發之動脈硬化發生機率，提高血液透析的安全性。
關鍵詞：血液透析，血液相容性，動脈硬化，氧化壓力，凝血

Abstract

The main therapeutic strategy for patient with end stage renal failure is to perform regularly hemodialytic process. The regularly hemodialytic process can remove uremic solutes from uremic patient, but the process also elevates the risk of atherosclerosis due to hemoincompatibility occurred between plasma and artificial kidney. The main purpose of this study is to investigate and compare the change of the four plasma atherogenic factors such as dityrosine, total antioxidant status (TAS), C-reactive protein (CRP) and tissue factor pathway inhibitors (TFPI) after performing hemodialysis for two groups of uremic patients with and without the supplement of green tea extract (GTE), respectively. The experimental data showed that the above four atherogenic factors in patient with supplement of GTE were closer to normal range than patient without supplement of GTE after performing hemodialytic process. These results suggest that supplement of GTE may reduce the risk of atherosclerosis associated with hemoincompatibility during uremic patient with hemodialytic process.
Keywords: hemodialysis, hemocompatibility, atherosclerosis, oxidative stress, coagulation

I. INTRODUCTION

Application of hemodialytic process to remove plasma uremic solutes in patient with end stage renal failure is necessary for the maintenance of life quality. However plasma metabolic wastes can be removed by hemodialysis, the hemoincompatibility is initiated due to the interaction between blood and artificial kidney [1-3].

Hemoincompatibility induced by hemodialysis can cause the imbalance of homeostasis, for example, activation of coagulation [4,5] and production of oxidative stress [6,7]. Both of above changes are causative agents for the development of atherosclerosis. This may illustrate why atherosclerosis would be one of the major complications in uremic patient [8-10]. In order to decrease the risk of atherogenic complication, supplement of green tea extract

[1] 中原大學生物醫學工程系 [2] 台灣血液淨化基金會
[*]Corresponding author. Email: yenlin@cycu.edu.tw
[1] Department of Biomedical Engineering, Chung Yuan Christian University, 200, Chung Pei Rd., Chung Li, Taiwan 32023, R.O.C.
[2] Formosan Blood Purification Foundation, 52, Pa-Teh Rd., Taipei, Taiwan 10058, R.O.C.
Manuscript received 12 March 2007; revised 22 May 2007; accepted 4 July 2007

(GTE) may be an excellent option for uremic patient to reduce oxidative damage caused by hemoincompatibility and decreases the risk of atherosclerosis [11,12].

The aim of this study is to investigate the relationships between GTE supplement and changes of atherogenic factors when hemodialytic process is performed for uremic patient. In addition to oxidative factors, the other two atherogenic factors such as inflammation and coagulation will be also observed after uremic patient with hemodialytic process to collect more information about GTE-treatment in the prevention of atherosclerosis induced by hemodialysis.

II. MATERIALS AND METHODS

Study subjects were divided into healthy, GET-treatment (uremic patients with GTE supplement) and placebo (uremic patients without GTE supplement) groups. Ten uremic patients (6 males and 4 females) were in GTE -treatment group with averaged hemodialytic duration of 85±23 months. GTE tablets (260mg, Numen Biotech. Co. Ltd., Taipei, Taiwan) given in GTE treatment group were 5 tablets and twice a day. The dosage of GTE did not impact on human health according to the studies of Ullmann et al [13] and Lee et al [14]. Ten uremic patients (6 males and 4 females) were in placebo group with averaged hemodialytic duration of 78±30 months. No GTE were given in placebo group during experimental period. Ten healthy volunteers (7 males and 3 females) were selected according to laboratory blood tests. The experiment was lasted for 3 months.

Plasma samples for experimental tests could be divided into 5 types. They were from healthy group (healthy), pre-hemodialysis of GTE-treatment group (HD1+C), post-hemodialysis of GTE-treatment group (HD2+C), pre-hemodialysis of placebo group (HD1) and post- hemodialysis of placebo group (HD2). Four atherogenic factors i.e. dityrosine, total antioxidant status (TAS), C-reactive protein (CRP) and tissue factor pathway inhibitor (TFPI) were determined for all plasma samples. Determination of TFPI for all plasma samples was achieved by commercial kit from America Diagnostica Inc. (500 West Avenue, Stamford, CT, USA). The other three factors (dityrosine, TAS and CRP) were determined by IMMULITE (DPC Cirrus Inc., Los Angles, CA, USA).

III. STATISTICAL ANALYSIS

All experimental data were expressed as mean±SD. Paired t test was applied to analyze the difference between two groups. Statistical difference between two groups was expressed as $p<0.05$.

IV. RESULTS

Dityrosine is one of the products after protein oxidized [15,16]. The higher plasma dityrosine detected, the higher atherogenic risk occurred. Figure 1 showed the results of plasma dityrosine for all plasma samples. We could find that both of dityrosine levels in HD1 and HD1+C were higher than healthy (healthy vs HD1 vs HD1+C=909±59 vs 6212±505 vs 4963±688, $p<0.05$). In addition, we could observe that dityrosine level of HD1+C was lower than HD1 (HD1 vs HD1+C=6212±505 vs 4963±688, $p<0.05$).

TAS represents the total antioxidant power of plasma [17,18]. TAS mainly includes antioxidant enzymes (SOD, catalase, GPx…etc) and small antioxidant molecules (GSH, Vitamin E, Vitamin C…etc). The atherogenic risk decreases when plasma TAS closes to normal range. Figure 2 showed the results of plasma TAS for all plasma samples. Both of Plasma TAS levels of HD1 and HD1+C were lower than healthy (healthy vs HD1 vs HD1+C=1.69±0.14 vs 1.10±0.11 vs 1.28±0.31, $p<0.05$). After hemodialysis, TAS level in HD2+C was higher than HD2 (HD2 vs HD2+C=0.77±0.13 vs 1.05±0.24, $p<0.05$).

CRP is one of useful markers for inflammatory and atherogenic evaluation [19-22]. It is nearly undetectable in healthy plasma. The higher plasma CRP detected, the higher atherogenic risk occurred. Figure 3 showed the results of plasma CRP for all plasma samples. No matter in

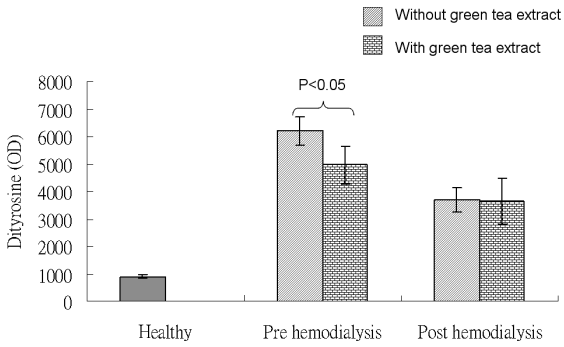

Figure 1 Determination of plasma dityrosine for all samples.

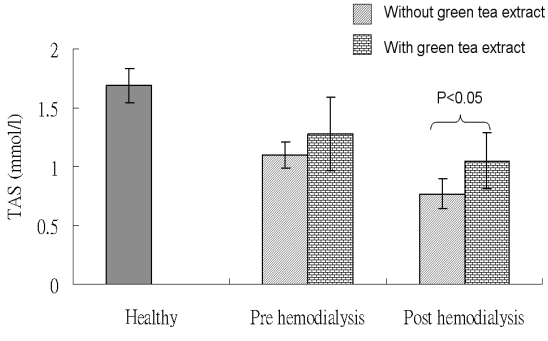

Figure 2 Determination of plasma TAS for all samples.

pre- or post- hemodialysis, CRP levels in group with GTE supplement were lower than those in group without GTE supplement (HD1 vs HD1+C=0.44±0.36 vs 0.05±0.02, $p<0.05$; HD2 vs HD2+C=0.57±0.45 vs 0.10±0.04, $p<0.05$).

The main function of TFPI is to inhibit the activation of coagulant factor X. In addition, elevated plasma TFPI level may be related to the development of glomerulonephritis [23], thrombosis and atherosclerosis in uremic patients [24]. So the plasma TFPI is great associated with the production of thrombosis and atherogenic development [25-27]. In figure 4, TFPI levels in group with GTE supplement were lower than those in group without GTE supplement (HD1 vs HD1+C=188±25 vs 153±26, $p<0.05$; HD2 vs HD2+C=222±40 vs 175±31, $p<0.05$).

V. DISCUSSION

The oxidative stress in the uremic patients are higher than in the healthy persons due to the result of hemoincompatibility induced by long-term hemodialytic process. Figure 1 showed the difference of oxidative stress between healthy and uremic groups (healthy vs. HD1, $p<0.05$). On the other hand, the oxidative stress in the uremic patients with GTE supplement was lower than the patients without GTE supplement (figure 1, HD1 vs. HD1+C, $p<0.05$). These results indicated that GTE could decrease oxidative stress in uremic patients.

The results of TAS in figure 2 were as similar as in figure 1. TAS in healthy group was higher than uremic group (figure 2, healthy vs. HD1, $p<0.05$). Although the pre-hemodialytic result of TAS in the uremic patients with and without GTE supplement was not statistical difference (figure 2, HD1 vs. HD1+C, $p=0.096$), the post-hemodialytic TAS result in the uremic patients with and without GTE supplement was statistical difference (figure 2, HD2 vs. HD2+C, $p<0.05$). These results suggested that GTE could increase antioxidant power against oxidative stress induced by hemoincompatibility in the uremic patients with long-term hemodialysis.

Inflammatory response is activated due to hemoincompatibility, so figure 3 illustrated why CRP in the uremic patients was higher than healthy subjects (healthy vs. HD1, $p<0.05$). On the other hand, CRP in the uremic patients with GTE supplement was lower than the patients without GTE supplement in both pre- and post- hemodialysis (figure 3, HD1 vs. HD1+C, $p<0.05$; HD2 vs. HD2+C, $p<0.05$). These results suggested that supplement of GTE in uremic patients could reduce inflammatory response induced by hemoincompatibility. These findings were consistent with previous studies [28,29].

TFPI is associated with the production of thrombosis. In figure 4, we could observe that plasma TFPI in the uremic patients was higher than healthy subjects due to long-term hemodialysis (healthy vs. HD1, $p<0.05$). Furthermore, TFPI in patients with GTE supplement were lower than patients without GTE supplement in both pre- and post-hemodialysis (figure 4, HD1 vs. HD1+C, $p<0.05$; HD2 vs. HD2+C, $p<0.05$). So figure 4 illustrated that supplement of GTE could reduce the formation of thrombosis induced by hemoincompatibility.

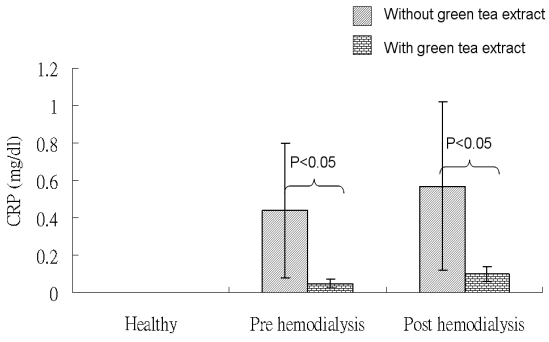

Figure 3 Determination of plasma CRP for all samples.

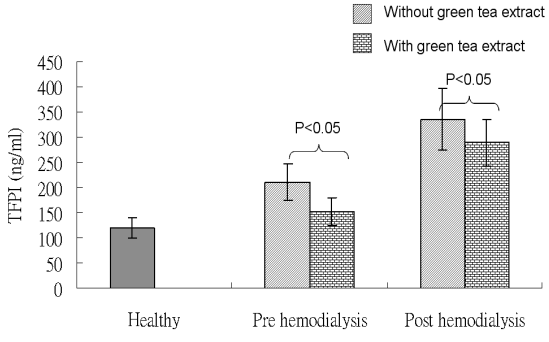

Figure 4 Determination of plasma TFPI for all samples.

VI. CONCLUSION

Hemodialytic process assists the uremic patients to remove uremic solutes in plasma, but this process causes the imbalance of homeostasis due to hemoincompatibility induced by hemodialysis. According to our experimental data, the four atherogenic factors such as dityrosine, TAS, CRP and TFPI were statistical differences between the uremic patients and healthy subjects. These results indicated that the risk of atherosclerosis in the uremic patients was higher than healthy subjects due to long-term hemodialysis. By contrast, no matter in pre- or post- hemodialysis, the four atherogenic factors in the uremic patients with GTE supplement were closer to normal ranges than patients without GTE supplement. Our study results may suggest that supplement of GTE in the uremic patients may decrease the risk of atherosclerosis induced by hemoincompatibility and improve the safety and efficiency of hemodialytic process.

ACKNOWLEDGEMENTS

We greatly thank Numen Biotech. Co. Ltd. (Taipei, Taiwan) and Formosan Blood Purification Foundation (Taipei, Taiwan) for technical and financial supports during experimental period.

REFERENCES

[1] G. Banche, V. Allizand, F. Giacchino, N. Mandras, J. Roana, F. Bonello, P. Belardi, V. Tullio, C. Merlino, N. Carlone and A. M. Cuffini, "Effect of dialysis membrane biocompatibility on polymorphonuclear granulocyte activity in dialysis patients," *Nephrol Dial Transplant*., vol. 21, pp. 3523-3528, Dec. 2006.

[2] E. Boulanger, O. Moranne, M. P. Wautier, J. P. Rougier, P. Ronco, D. Pagniez and J. L. Wautier, "Biocompatibility of peritoneal dialysis fluids," *Nephrol Ther.*, vol. 1, pp. 14-22, Mar. 2005.

[3] H. D. Humes, W. H. Fisell and K. Tiranathanagul, "The future of hemodialysis membranes," *Kidney Int.*, vol. 69, pp. 1115-1119, Apr. 2006.

[4] M. Sabovic, B. Salobir, I. Preloznik Zupan, P. Bratina, V. Bojec and J. Buturovic Ponikvar, "The influence of the haemodialysis procedure on platelets, coagulation and fibrinolysis," *Pathophysiol Haemost Thromb*., vol. 34, pp. 274-278, May 2005.

[5] K. Jr. Opatrny, A. Krouzzecky, K. Polanska, J. Mares, M. Tomsu, S. K. Bowry and J. Vienken, "Does an alteration of dialyzer design and geometry affect biocompatibility parameters?" *Hemodial Int*., vol. 10, pp. 201-208, Apr. 2006.

[6] J. Borawski, "Myeloperoxidase as a marker of hemodialysis biocompatibility and oxidative stress: the underestimated modifying effects of heparin," *Am J Kidney Dis.*, vol. 47, pp. 37-41, Jan. 2006. Review.

[7] J. Himmelfarb and R. M. Hakim, "Oxidative stress in uremia," *Curr Opin Nephrol Hypertens.,* vol. 12, pp. 593-598, Nov. 2003. Review.

[8] O. J. Guzon and K. C. Dellsperger, "Cardiovascular issues in dialysis patients: challenges and newer insights," *Adv Perit Dial*., vol. 22, pp. 111-115, Dec. 2006. Review.

[9] E. Franek, R. Blaschyk, A. Kolonka, L. Mazur-Psonka, H. Langowska-Adamczyk, F. Kokot and A. Wiecek, "Chronic periodontitis in hemodialysis patients with chronic kidney disease is associated with elevated serum C-reactive protein concentration and greater intima-media thickness of the carotid artery," *J Nephrol*., vol. 19, pp. 346-351, May 2006.

[10] I. Shahab and K. D. Nolph, "MIA syndrome in peritoneal dialysis: prevention and treatment," *Contrib Nephrol*., vol. 150, pp. 135-143, Dec. 2006. Review.

[11] T. Nakagawa, T. Yokozaya, M. Sano, S. Takeuchi, M. Kim and S. Minamoto, "Activity of (-)-epigallocatechin 3-O-gallate against oxidative stress in rats with adenine-induced renal failure," *J Agric Food Chem.*, vol. 52, pp. 2103-2107, Apr. 2004.

[12] T. Yokozawa, E. J. Cho and T. Nakagawa, "Influence of green tea polyphenol in rats with arginine-induced renal failure," *J Agric Food Chem.*, vol. 51, pp. 2421-2425, Apr. 2003.

[13] U. Ullmann, J. Haller, J. P. Decourt, N. Girault, J. Girault, A. S. Richard Caudron, B. Pineau and P. Weber, "Asingle ascending dose study of epigallocatechin gallate in healthy volunteers," *J Int Med Res*., vol. 31, pp. 88-101, Mar. 2003.

[14] M. J. Lee, P. Maliakal, L. Chen, X. Meng, F. Y. Bondoc, S. Prabhu, G. Lambert, S. Mohr and C. S. Yang, "Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability," Cancer Epidemiol Biomarkers Prev., vol. 11, pp. 1025-1032, Oct. 2000.

[15] K. C. Huang, C. C. Yang, K. T. Lee and C. T. Chien, "Reduced hemodialysis-induced oxidative stress in end-stage renal disease patients by electrolyzed reduced water," *Kidney Int*., vol. 64, pp. 704-714, Aug. 2003.

[16] B. Tovbin, D. Mazor, M. Vorobiov, C. Chaimovitz and N. Meyerstein, "Induction of protein oxidation by intravenous iron in hemodialysis patients: role of inflammation," *Am J Kidney Dis*., vol. 40, pp. 1005-1012, Nov. 2002.

[17] G. Mirescu, C. Capusa, I. Stoian, B. Vargolici, B. Barbulescu and N. Ursea, "Global assessment of serum antioxidant status in hemodialysis patients," *J Nephrol*., vol. 18, pp. 599-605, Sep. 2005.

[18] B. Mayer, S. Zitta, J. Greilberger, H. Holzer, G. Geibnegger, A. Hermetter and K. Oettl, "Effect of hemodialysis on the antioxidative properties of serum," *Biochim Biophys Acta*., vol. 1638, pp. 267-272, Jul. 2003.

[19] M. Kanwar, M. Hashem, H. Rosman, D. Kamalakannan, A. Cheeman, A. Ali, J. Gardin and J. J. Maciejko, "Usefulness of clinical evaluation, troponins, and C-reactive protein in predicting mortality among stable hemodialysis patients," *Am J Cardiol.*, vol. 98, pp. 1283-1287, Nov. 2006.

[20] R. Bbreiterman-White, "C-reactive protein and anemia: implications for patients on dialysis," *Nephrol Nurs J.*, vol. 33, pp. 555-558, Sep. 2006. Review.

[21] M. El-Khatib, H. J. Duncan and K. S. Kant, "Role of C-reactive protein, reticulocyte haemoglobin content and inflammatory markers in iron and erythropoietin administration in dialysis patients," *Nephrology (Carlton).,* vol. 11, pp. 400-104, Oct. 2006.

[22] S. Racki, L. Zaputovic, Z. Mavirc, B. Vujicic and S. Dvornik, "C-reactive protein is a strong predictor of mortality in hemodialysis patients," *Ren Fail.*, vol. 28, pp. 427-433, Jun. 2006.

[23] B. A. Lwaleed and P. S. Bass, "Tissue factor pathway inhibitor: structure, biology and involvement of disease," J Pathol., vol. 208, pp. 327-339, 2006.

[24] P. Zemanova, K. Opatry, S. Opatrna, L. Vit, F. Sofrna and J. Racek, "Tissue factor, its pathway inhibitor, and metabolic disturbances in long-term peritoneal dialysis," *Kidney Blood Press Res*, vol. 26, pp. 368-375, Nov. 2003.

[25] P. Zemanova, K. Opatrny, L. Vit and F. Sefrna, "Tissue factor, its inhibitor, and the thrombogenicity of two new synthetic membranes," *Artif Organs.*, vol. 29, pp. 651-657, Aug. 2005.

[26] K. Pawlak, D. Pawlak and M. Misliwiec, "Extrinsic coagulation pathway activation and metalloproteinase-2/TIMPs system are related to oxidative stress and atherosclerosis in hemodialysis patients," *Thromb Haemost.*, vol. 92, pp. 646-653, Sep. 2004.

[27] P. Zemanova, K. Jr. Opatrny, S. Opatrna, L. Vit, F. Sefrna and J. Racek, "Tissue factor, its pathway inhibitor, and metabolic disturbances in long-term peritoneal dialysis," *Kidney Blood Press Res.*, vol. 26, pp. 368-375, Jan. 2003.

[28] A. Steptoe, E. L. Gibson, R. Vounonvirta, M. Hamer, J. Waedle, J. A. Martin and J. D. Erusalimsky, "The effects of chronic tea intake on platelet activation and inflammation: A double-blind placebo controlled trial," *Atherosclerosis*, vol. 28, 2006. Article in press.

[29] D. De Backer, E. Clay, J. Delaughe and G. De Backer, "Epidemiological evidence for an association between habitual tea consumption and markers of chronic inflammation," *Atherosclerosis*, vol. 189, pp. 428-435, Dec. 2006.