Antioxidants in the treatment of patients with renal failure

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Abstract

Renal failure is accompanied by oxidative stress, which is caused by enhanced production of reactive oxygen species and impaired antioxidant defense. The suggested therapeutical interventions aimed at reducing oxidative stress in chronic renal failure patients are as follows: 1) the use of biocompatible membranes, ultrapure dialysate, and removal of endogenous foci of infection; 2) haemolipodialysis, and electrolysed reduced water for dialysate preparation; 3) administration of antioxidants (α-tocopherol, ascorbic acid, N-acetylcysteine, reduced glutathione); 4) substances possibly affecting oxidative stress indirectly (erythropoietin, sodium selenite). As currently available data have, as yet, provided rather limited evidence for the clinical benefit of antioxidant interventions, at present it is untimely to give practical recommendations with regard to antioxidant treatment of patients with renal failure.

Key words: renal failure, oxidative stress, antioxidant treatment.

Introduction

Renal failure is accompanied by oxidative stress [1,2], which consists in the damage of biological structures by reactive oxygen species due to their excessive generation and impaired efficiency of antioxidant defense mechanisms.

In renal failure patients enhanced reactive oxygen species production is underlain mainly by inflammation [3,4], malnutrition [3], presence of endogenous stable oxidants in the uraemic plasma [5]. In haemodialysis patients the additional stimulus for increased free oxygen radical production can be the haemodialysis procedure itself [6,7]. It is mainly due to inflammatory cell activation caused by insufficiently biocompatible membranes, which is amplified by various bacterial products passing across from the dialysate to the blood compartment [8,9]. Advanced age and diabetes are further factors increasing pro-oxidant activity in renal failure patients [4]. At the same time, impaired activities of endogenous enzymatic free radical scavengers (superoxide dismutase, glutathione peroxidase, catalase) and deficiency of non-enzymatic antioxidants (reduced glutathione, α-tocopherol, ascorbic acid, transferrin, albumin, 17β-oestradiol) aggravate the oxidative stress [1]. Moreover, antioxidant defenses insufficient to shut down the oxidative stress can lead to a chronic and vicious cycle of free radicals causing production of inflammatory mediators that in turn amplify the generation of reactive oxygen species. Either chronic or acute production of free radicals leads to the oxidative modification of lipids, arachidonic acid derivatives, carbohydrates, amino acids, proteins, and deoxyribonucleic acid [1]. In addition, it activates cellular signaling events regulating cell division [10], differentiation [11], and apoptosis [12].

There is growing evidence from experimental and clinical studies that in chronic renal failure oxidative stress can be considered as a potentially important source of patient morbidity and mortality. It may be implicated in the pathogenesis of atherosclerosis [2,13,14], malnutrition [2,13,14], anaemia [15], dialysis-induced amyloidosis [16], and possibly increased risk of cancerogenesis [17] in these patients. Therefore, for some time past various therapeutic interventions have been attempted in order to reduce oxidative stress in chronic renal failure in the hope to improve patient outcome. Therapeutic approaches to reduce oxidative stress in chronic renal failure patients

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Received 4.05.2004 Accepted 11.06.2004

Table 1. **Directions of therapeutic interventions considered for reducing oxidative stress in chronic renal failure patients**

A. Reduction of inflammatory cell activation:
1) biocompatible dialysis membranes;
2) ultrapure dialysate;
3) removal of focal infections.

B. Removal of inflammatory mediators:
1) haemolipodialysis;
2) electrolysed reduced water for dialysate preparation.

C. Administration of antioxidants:
1) α-tocopherol;
2) ascorbic acid;
3) N-acetylcysteine;
4) reduced glutathione.

D. Substances possibly affecting oxidative stress indirectly:
1) erythropoietin (?);
2) sodium selenite (?).

are focused on the reduction of inflammatory cell activation, removal of inflammatory mediators and the use of antioxidants [8,9]. Measures considered to date to accomplish this aim are presented in *Tab. 1*.

Reduction of inflammatory cell activation

Reduction of inflammatory cell activation can be attained by the use of biocompatible dialysis membranes, ultrapure dialysate, and removal of focal infections.

Biocompatible dialysis membranes. Haemodialysis procedure performed with a biocompatible (e.g. polyacrylonitrile or polysulfone) dialysis membranes is known to reduce significantly intradialytic oxidative stress [7]. Therefore, one can expect that decreased production of active oxygen species would prevent overproduction of oxidized LDL and enhanced endothelial dysfunction, and, consequently, the risk of atherosclerosis with subsequent cardiovascular complications would be reduced. However, this has not, as yet, been confirmed conclusively, and convincing proofs for cause-effect relationship between membrane biocompatibility per se and accelerated atherosclerosis are still lacking.

Ultrapure dialysate. Reactive oxygen species were found to induce oxidative modification of β_2-microglobulin amyloidosis, and thus, to favour the development of dialysis-induced amyloidosis [16]. Long-term observations disclosed that regular haemodialysis treatment with ultrapure dialysate was associated with a significant decrease in amyloidosis-induced carpal tunnel syndrome compared with pure dialysate [18]. However, it is not, as yet, known whether this phenomenon was affected by the reduction of intradialytic reactive oxygen species production with ultrapure dialysate only or by other factors.

Removal of focal infections. As infections induce oxidative burst of inflammatory cells, removal of focal (i.e. dental, tonsillar, and other) infections, and preventive measures against vascular access infections are of importance.

Removal of inflammatory mediators

Among suggested measures aimed at removal of inflammatory mediators there are haemolipodialysis, and electrolysed reduced water.

Haemolipodialysis relies on the addition of liposomes to the dialysate during the standard haemodialysis procedure [8,9]. The liposomes of 250-300 nm in diameter are comprised of lyophilized soybean phosphatidylcholine bilayer with incorporated α-tocopherol, which form a unilamellar bilayer upon addition to dialysate [8,9]. At the same time, water-soluble ascorbic acid is added directly to the dialysate. These two antioxidants are used together to increase removal of hydrophobic toxins and inflammatory mediators on the one side, and to synergistically scavenge oxidants on the other side, thereby supporting the host's antioxidant defense system. Maintenance of the appropriate ratio between α-tocopherol and ascorbic acid is of crucial importance to avoid their pro-oxidant action. The preliminary experience with haemolipodialysis for the prevention of intradialytic oxidative stress is, however limited, though promising [9].

Electrolysed reduced water. During electrolysis of raw water the active atomic hydrogen with higher reducing activity is released on the cathode. Administration of the dialysate prepared from this electrolysed reduced water during haemodialysis efficiently scavenged hydrogen peroxide and hypochlorite, and ameliorated antioxidant status during one-month treatment [19].

Antioxidants

Among antioxidants α-tocopherol, ascorbic acid, N-acetylcysteine, and reduced glutathione were tried to modify oxidative stress in renal failure.

A-tocopherol. To date, α-tocopherol (vitamin E) was the most frequently used antioxidant to achieve adequate control of oxidative stress in chronic renal failure patients. Short- or long-term administration of vitamin E orally or intramuscularly has been reported to modify beneficially their oxidative status [20-26]. In addition, haemodialysis procedures performed with vitamin E bonded membrane not only ameliorated antioxidative defense [27-33], but also significantly reduced the percentage increase of the aortic calcification index [30], improved carotid intima-media thickness, viscosity and dysmorphism of the red blood cells [30], and haemolysis [32] with resultant reduction of erythropoietin requirements for the treatment of uraemic anaemia [29]. Haemodialysis by vitamin E-coated membrane also prevented dialysis-induced endothelial dysfunction [33].

Although observational studies suggested possible beneficial effect of vitamin E on cardiovascular complications, except for the CHAOS study [34] the vast majority of large placebo-controlled studies on non-renal population (GISSI [35], HOPE [36], SECURE [37], HPS [38]) were rather discouraging,

because they failed to demonstrate a positive effect of vitamin E on cardiovascular event rates.

Meanwhile, the first double-blind placebo-controlled randomized SPACE study [39] performed on 196 haemodialysed patients disclosed a significant decrease in combined cardiovascular event rates in the group of orally treated with vitamin E 800 IU/day over 2 years, but no significant differences in overall mortality and mortality from cardiovascular disease were observed. However, in view of negative influence of vitamin E on the blood serum level of protective HDL_2 cholesterol, its safety in the long-term antioxidative treatment needs to be determined [2].

Ascorbic acid represents one of the most prominent antioxidants, exerting beneficial effects by an inhibition of lipid peroxidation and by reducing endothelial dysfunction [40]. Although in chronic renal failure patients deficiency of vitamin C can be observed, its administration in these patients requires deliberation. Vitamin C in food or as supplementation may lead to its excessive serum levels, resulting in hyperoxalaemia that may contribute to vascular disease in uraemic patients [41]. In addition, in the presence of transition metals like iron, ascorbate may give rise to an increased generation of antioxidants, and ascorbylation may impose additional carbonyl stress to uraemic patients, particularly in the presence of high blood glucose levels [40]. Therefore, 60 mg of oral vitamin C are currently recommended for chronic kidney patients [42], while in case of suspected subclinical ascorbate deficiency 1-1.5 g of oral vitamin C per week or 300 mg parenteral ascorbate per dialysis session are recommended, respectively [43].

N-acetylcysteine. In the recently published randomized controlled trial [44], treatment with the reduced thiol-containing antioxidant N-acetylcysteine (600 mg orally twice a day for a median of 14.5 months) significantly reduced cardiovascular events by 40% in the treated group compared with the placebo group. However, no effect was reported on total or cardiovascular mortality.

Reduced glutathione. Intravenous administration of exogenous reduced glutathione (tationil) alone [45] or in combination with the use of vitamin E bonded dialysis membrane [46] significantly improved uraemic anaemia. Some investigators [46] believe that combined use of the vitamin E bonded membrane and intravenous reduced glutathione seems to be the best antioxidant therapy so far, with significant saving of recombinant human erythropoietin dose.

Substances with possible indirect antioxidant action

Also some substances with possible indirect antioxidant action, e.g. erythropoietin, and sodium selenite, seem to be helpful for antioxidant treatment in renal failure.

Though erythropoietin is not an antioxidant, it is suggested that in chronic renal failure it can reduce oxidative stress indirectly by the correction of uraemic anaemia, and consequently, the rise in glutathione content in the blood. However, up-to-date reported studies did not provide univocal results. During recombinant human erythropoietin therapy some authors did not found significant changes in the oxidative stress intensity [47] or antioxidant status [48], while others observed positive effects [49-51].

Selenium deficiency, frequently observed in chronic renal failure patients [48], may contribute to the impairment of activity of glutathione peroxidase, an enzymatic antioxidant belonging to selenoproteins. Correction of selenium deficiency by sodium selenite given intravenously [52] significantly increased red blood cell glutathione peroxidase activity, while selene-rich yeasts given orally did not affect this enzyme activity [53]. Influence of selenite supplementation on other components of the antioxidant system remains unclear.

Conclusions

There are many prerequisites suggesting possible beneficial effects of therapeutic interventions aimed at reducing oxidative stress in chronic renal failure, and the recently published results of two randomised placebo-controlled clinical trials [39,44] are particularly promising in this respect. However, according to Steinberg and Witztum [54] several crucial questions still remain unanswered: 1) have the clinical trials been done with the right antioxidants at the right doses? 2) was the effectiveness of these antioxidants investigated properly? 3) are the right markers for oxidative stress and for the effectiveness of the antioxidants identified? 4) were the patients for the antioxidative therapies chosen accurately? 5) have the trials been started early enough, and have they lasted long enough? 6) are the species differences such that the results in animal models do not extrapolate to humans? Answering these questions will enable to design suitable antioxidant protocols and to evaluate their effectiveness in chronic renal failure. As currently available data have, as yet, provided rather limited evidence for clinical benefit of antioxidant interventions, at present it is untimely to give practical recommendations with regard to antioxidant treatment of patients with renal failure.

References

1. Himmelfarb J, Hakim RM. Oxidative stress in uremia. Curr Opin Nephrol Hypertens, 2003; 12: 593-8.
2. Galle J. Oxidative stress in chronic renal failure. Nephrol Dial Transplant, 2001; 16: 2135-7.
3. Stenvinkel P, Heimbürger O, Paultre F. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney Int, 1999; 55: 1899-911.
4. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrol Dial Transplant, 2003; 18: 1272-80.
5. Roselaar SE, Nazhat NB, Winyard PG, Jones P, Cunningham J, Blake DR. Detection of oxidants in uremic plasma by electron spin resonance spectroscopy. Kidney Int, 1995; 48: 199-206.
6. Trznadel K, Pawlicki L, Kędziora J, Luciak M, Błaszczyk J, Buczyński A. Superoxide anion generation, erythrocyte superoxide dismutase activity, and lipid peroxidation during hemoperfusion and haemodialysis in chronic uremic patients. Free Radic Biol Med, 1989; 6: 393-7.
7. Luciak M, Trznadel K. Free oxygen species metabolism during haemodialysis with different membranes. Nephrol Dial Transplant, 1991; 6(3): 66-70.
8. Wratten ML, Navino C, Tetta C, Verzetti G. Haemolipodialysis. Blood Purif, 1999; 17: 127-33.
9. Wratten ML, Tetta C, Ursini F, Sevanian A. Oxidant stress in

hemodialysis: prevention and treatment strategies. Kidney Int, 2000; 58(76): 126-32.

10. Bae GU, Seo DW, Kwon HK, Lee HY, Hong S, Lee ZW, Ha KS, Lee HW, Han JW. Hydrogen peroxide activates p70 (S6k) signaling pathway. J Biol Chem, 1999; 274(32): 596-602.

11. Satter M, Winkler T, Verma S, Byrne CH, Shrikhande G, Salgia R, Griffin JD. Hematopoietic growth factors signal through the formation of reactive oxygen species. Blood, 1999; 93: 2928-35.

12. Goossens V, de Vos K, Vercammen D, Steemans M, Vancompernolle K, Fiers W, Vandenabeele P, Grooten J. Redox regulation of TNF signalling. Biofactors, 1999; 10: 145-56.

13. Maggi E, Belazzi R, Falaschi F, Frattoni A, Perani G, Finardi G, Gazo A, Nai M, Romanini D, Bellono G. Enhanced LDL oxidation in uremic patients: an additional mechanism for accelerated atherosclerosis? Kidney Int, 1994; 45: 867-83.

14. Galle J, Seibold S, Wanner C. Inflammation in uremic patients: what is the link? Kidney Blood Press Res, 2003; 26: 65-75.

15. Taccone-Gallucci M, Lubrano R, Meloni C, Morosetti M, Manca di Villahermosa S, Scoppi P, Palombo G, Castello M, Casciani C. Red blood cell membrane lipid peroxidation and resistance to erythropoietin therapy in hemodialysis patients. Clin Nephrol, 1999; 52: 239-45.

16. Cappeillère-Blandin C, Deleveau T, Descamps-Latscha B. Structural modifications of human beta-2 microglobulin treated with oxygen-derived radicals. Biochem J, 1991; 277: 175-82.

17. Vamvakas S, Bahner U, Heidland A. Cancer in end-stage renal disease: potential factors involved. Am J Nephrol, 1998; 18: 89-95.

18. Berland Y, Brunet P, Ragon A, Reynier JP. Dialysis fluid and water: their roles in biocompatibility. Nephrol Dial Transplant, 1995; 10(10): 45-7.

19. Huang KC, Yang CC, Lee KT, Chien CT. Reduced hemodialysis-induced oxidative stress in end-stage renal disease patients by electrolyzed reduced water. Kidney Int, 2003; 64: 704-14.

20. Giardini O, Taccone-Gallucci M, Lubrano R, Ricciardi-Tenore G, Bandino D, Silvi L, Paradisi C, Mannarino O, Citti G, Elli M, Casciani CU. Effects of alpha-tocopherol administration on red blood cell membrane lipid peroxidation in hemodialysis patients. Clin Nephrol, 1984; 21: 174-7.

21. Taccone-Gallucci M, Lubrano R, Del Principe D, Menichelli A, Giordani M, Citti G, Morosetti M, Meloni C, Mazzarella V, Meschini L, Tozzo C, Elli M, Giardini O, Casciani CU. Platelet lipid peroxidation in haemodialysis patients: effects of vitamin E supplementation. Nephrol. Dial. Transplant, 1989; 4: 975-8.

22. Lubrano R, Taccone-Gallucci M, Piazza A, Morosetti M, Meloni C, Citti G, Mannarino O, Castello MA, Casciani CU. Vitamin E supplementation and oxidative status of peripheral blood mononuclear cells and lymphocyte subsets in hemodialysis patients. Nutrition, 1992; 8: 94-7.

23. Yukawa S, Hibino A, Maeda T, Mimura K, Yukawa A, Maeda A, Kishino M, Sonobe M, Mune M, Yamada Y, Niside I. Effect of alpha-tocopherol on in vitro and in vivo metabolism of low-density lipoproteins in haemodialysis patients. Nephrol Dial Transplant, 1995; 10(3): 1-3.

24. Hassan MQ, Hussain SA, Zaki MA, Alsharif NZ, Stohs SJ. Protective effects of antioxidants against uraemia-induced lipid peroxidation and glutathione depletion in humans. Pharmacol Toxicol, 1995; 77: 407-11.

25. Yalçin AS, Yurtkuran M, Dilek K, Kilinç A, Taga Y, Emerk K. The effect of vitamin E therapy on plasma and erythrocyte lipid peroxidation in chronic hemodialysis patients. Clin Chim Acta, 1989; 185: 109-12.

26. Cristol JP, Bosc JY, Badiou S, Leblanc M. Lorrho R, Descomps B, Canaud B. Erythropoietin and oxidative stress in haemodialysis: beneficial effects of vitamin E supplementation. Nephrol Dial Transplant, 1997; 12: 2312-7.

27. Buoncristiani U, Galli F, Rovidati S, Albertini MC, Campus G, Canestrari F. Oxidative damage during hemodialysis using a vitamin-E-modified dialysis membrane: a preliminary characterization. Nephron, 1997; 77: 57-61.

28. Tarng DC, Huang TP, Liu TY, Chen HW, Sung YJ, Wei YH. Effect of vitamin E-bonded membrane on the 8-hydroxy-2'-deoxyguanosine level in leukocyte DNA of hemodialysis patients. Kidney Int, 2000; 58: 790-9.

29. Usberti M, Gerardi GM, Bufano G, Tira P, Micheli A, Albertini M, Floridi A, Di Lorenzo D, Galli F. Effects of erythropoietin (EPO) and vitamin E-modified membrane (CL-E) on plasma oxidative stress markers and anemia of hemodialyzed patients. Am J Kidney Dis, 2002; 40: 590-9.

30. Kobayashi S, Moriya H, Aso K, Ohtake T. Vitamin E-bonded hemodialyzer improves atherosclerosis associated with a rheological improvement of circulating red blood cells. Kidney Int, 2003; 63: 1881-7.

31. Mune M, Yukawa S, Kishino M, Otani H, Kimura K, Nishikawa O, Takahashi T, Kodama N, Saika Y, Yamada Y. Effect of vitamin E on lipid metabolism and atherosclerosis in ESRD patients. Kidney Int, 1999; 56(71): 126-9.

32. Taccone-Gallucci M, Meloni C, Lubrano R, Morosetti M, Palombo G, Cianciulli P, Scoppi P, Castello MA, Casciani CU. Chronic hemolysis and erythrocyte survival in hemodialysis patients treated with vitamin E-modified dialysis filters. Contrib Nephrol, 1999; 127: 44-8.

33. Miyazaki H, Matsuoka H, Itabe H, Usui M, Ueda S, Okuda S, Imaizumi T. Hemodialysis impairs endothelial function via oxidative stress: effects of vitamin E-coated dialyzer. Circulation, 2000; 101: 1002-6.

34. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Matchison MJ, Brown MJ. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). Lancet, 1996; 347: 781-6.

35. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet, 1999; 354: 447-55.

36. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study investigations. New Engl J Med, 2000; 342: 154-60.

37. Lonn E, Yusuf S, Dzavik V, Doris I, Yi Q, Smith S, Moore-Cox A, Bosch J, Riley WA, Teo KK. Effects of ramipril and vitamin E on atherosclerosis: the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). Circulation, 2001; 103: 919-25.

38. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet, 2002; 360; 23-33.

39. Boaz M, Smetana S, Weinstein T, Matas Z, Gafter U, Iaina A, Knecht A, Weissgarten Y, Brunner D, Fainaru M, Green MS. Secondary prevention with antioxidants of cardiovascular disease in end-stage renal disease (SPACE): randomized placebo-controlled trial. Lancet, 2000; 356: 1213-8.

40. Deicher R, Hörl WH. Vitamin C in chronic kidney disease and hemodialysis patients. Kidney Blood Press Res, 2003; 26: 100-6.

41. Pru C, Eaton J, Kjellstrand C. Vitamin C intoxication and hyperoxalaemia in chronic hemodialysis patients. Nephron, 1985; 39: 112-6.

42. Makoff R. Vitamin replacement therapy in renal failure patients. Miner Electrolyte Metab, 1999; 25: 349-51.

43. Hörl WH. Is there a role for adjuvant therapy in patients being treated with epoetin? Nephrol Dial Transplant, 1999; 14(2): 50-60.

44. Tepel M, van der Giet M, Statz M, Jankowski J, Zidek W. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. Circulation, 2003; 107: 992-5.

45. Costagliola C, Romano L, Scibelli G, de Vincentis A, Sorice P, di Benedetto A. Anemia and chronic renal failure: a therapeutical approach by reduced glutathione parenteral administration. Nephron, 1992; 61: 404-8.

46. Usberti M, Gerardi GM, Micheli AM, Tira P, Bufano G, Gaggia P, Movilli E, Cancarini GC, De Marinis S, D'Aviolo G, Broccoli R, Manganoni A, Albertini A, Di Lorenzo D. Effects of a vitamin E-bonded membrane and of glutathione on anemia and erythropoietin requirements in hemodialysis patients. J Nephrol, 2002; 15: 558-64.

47. Bozfakioglu S, Alptekin N, Seckin S, Ark E, Kocak-Toker N. Red cell lipid peroxidation and antioxidant system in chronic renal failure patients treated with recombinant human erythropoietin. Nephron, 1992; 61: 228-9.

48. Zachara BA, Adamowicz A, Trafikowska U, Trafikowska A, Manitius J, Nartowicz E. Selenium and glutathione levels, and glutathione peroxidase activities in blood components of uremic patients on hemodialysis supplemented with selenium and treated with erythropoietin. J Trace Elem Med Biol, 2001; 15: 201-8.

49. Sommerburg O, Grune T, Hampl H, Riedel E, van Kuijk FJMG, Ehrich JHH, Siems WG. Does long-term treatment of renal anaemia with recombinant erythropoietin influence oxidative stress in haemodialysis patients? Nephrol Dial Transplant, 1998; 13: 2583-7.

50. Delmas-Beauvieux MC, Combe C, Peuchant E, Carbonneau AN, Dubourg L, de Précigout V, Aparicio M, Clerc M. Evaluation

of red blood cell lipoperoxidation in hemodialysed patients during erythropoietin therapy supplemented or not with iron. Nephron, 1995; 69: 404-10.

51. Mimić-Oka J, Simić T, Djukanović L. Epoetin treatment improves red blood cell and plasma antioxidant capacity in hemodialysis patients. Renal Failure, 2002; 24: 77-87.

52. Koenig JS, Fischer M, Bulant E, Tiran B, Elmadfa I, Druml W. Antioxidant status in patients on chronic hemodialysis therapy: impact of parenteral selenium supplementation. Wien Klin Wochenschr, 1997; 109: 13-9.

53. Zachara BA, Trafikowska U, Adamowicz A, Nartowicz E, Manitius J. Selenium, glutathione peroxidases, and some other antioxidant parameters in blood of patients with chronic renal failure. J Trace Elem Biol Med, 2001; 15: 161-6.

54. Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? Circulation, 2002; 105: 2107-11.