Transpl Int (2003) 16: 53–57
DOI 10.1007/s00147-002-0495-9

BRIEF REPORT

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Fluvastin therapy affects TAFI concentration in kidney transplant recipients

Received: 9 October 2001
Revised: 4 July 2002
Accepted: 22 August 2002
Published online: 10 December 2002

The study was supported in part by a grant from the Polish Research Committee KBN (no. 6 PO 5 8 117 21).

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Abstract Thrombin activatable fibrinolysis inhibitor (TAFI) is a glycoprotein, linking coagulation and fibrinolysis. Recently, attention has been drawn to the beneficial effects of statins on haemostasis in kidney patients prone to dyslipidaemia and with a high risk of cardiovascular death. The purpose of this study was to assess whether fluvastatin affects TAFI concentration in renal transplant recipients. We evaluated thrombin–antithrombin (TAT) complexes, prothrombin fragments 1 + 2, thrombomodulin, plasmin–antiplasmin (PAP) complexes, TAFI, P-selectin, and lipoprotein (a), 1, 2, and 3 months before and after fluvastatin treatment and in normolipaemic kidney transplant recipients and healthy volunteers. Cholesterol and LDL fell significantly as soon as 1 month after treatment had begun and remained lowered during the therapy. TAFI and prothrombin fragments 1 + 2 decreased significantly after 3 months of fluvastatin administration, whereas P-selectin decreased significantly after 2 months and remained significantly lower after 3 months of this therapy. We can conclude that fluvastatin is an effective hypolipaemic agent that favourably affects haemostasis.

Keywords Haemostasis · Fluvastatin · TAFI · Kidney transplantation · Hyperlipidaemia

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in uraemic patients undergoing renal replacement therapy [1]. In kidney transplant recipients, the incidence of myocardial infarction is approximately 3–5 times higher than in the general population. Some statistics show that even 50–60% of deaths are directly attributable to cardiovascular diseases [2]. In addition to factors such as hypertension, impaired glucose tolerance, altered calcium levels, phosphate homeostasis, and a sedentary lifestyle, that contribute to the development of vascular disease in dialysed patients, there is an increased prevalence of hyperlipidaemia following renal transplantation [3], which further exacerbates the pre-existing atherogenic lipid profile. It is appreciated that hyperlipidaemia itself has a deleterious effect on graft function [4]. Treatment with cyclosporin A (CyA) is also associated with the increased risk of thrombo-embolic complications, cardiovascular morbidity, and progression of atherosclerosis. The importance of hyperlipidaemia and thrombosis in the development of ischaemic heart disease is well established. It is possible that prolonged hyperlipidaemia may adversely affect haemostasis, causing a prothrombotic state. Thrombin Activatable Fibrinolysis Inhibitor (TAFI) is a recently discovered glycoprotein that couples two functionally opposite systems: coagulation and fibrinolysis [5]. It is present in plasma as a proenzyme, which is converted to its active form, TAFIa, by thrombin. TAFIa removes COOH-terminal lysine and arginine residues from fibrin,

impairing formation of t-PA, plasminogen, and fibrin complex. It makes plasmin generation less effective [6]. At high concentrations, TAFI is a plasmin inhibitor, too [6]. Thrombomodulin in adequate concentration (soluble, as well as in cellular form), which catalyses the above process, plays the key role in activation of TAFI by thrombin [7]. HMG-CoA reductase inhibitors have been proven effective in treatment of hypercholesterolaemia. It has been reported that fluvastatin decreases soluble thrombomodulin concentration in cardiac transplant recipients [8]. To date there are, as far as we know, no data on the effect of statins on TAFI in kidney transplant recipients.

Materials and methods

The studies were performed on 12 renal allograft recipients with hyperlipidaemia (five women, seven men, age range 39–63 years). The immunosuppressive regimen was CyA (3.1 ± 1.2 mg/kg b.w., CyA trough levels 100–200 ng/ml), prednisone (7.5–10 mg daily) and azathioprine (100–150 mg daily). The patients had been engrafted for a period of 2–8 years. They all maintained sufficient and stable graft function and showed no clinical signs of rejection. The local ethics committee approved the study, and informed consent was obtained from all patients. Inclusion criteria were: hypercholesterolaemia (total cholesterol over 220 mg/dl, LDL over 160 mg/dl), no inflammation (C-reactive protein within normal range), and no liver dysfunction (prothrombin time, alanine aminotransferase within normal range).

Fluvastatin (Lescol, Novartis) was administered at a dose of 20 mg at bedtime for 3 months. Blood was drawn in the morning between 8.00 and 9.00 a.m. to avoid circadian variation [9] when patients appeared for routine office assessment after an overnight fast. Blood was taken without stasis. Venous blood samples were collected into 3.8% sodium citrate in a 9:1 volume ratio. The blood was centrifuged at 1,900 *g* for 20 min at room temperature to yield platelet-poor plasma (PPP). Samples were divided into aliquots and stored at –40 °C before assay.

We also studied normolipaemic kidney allograft recipients (five female, 16 male, age range 29–64 years) They were treated with the following immunosuppressive regimen: CyA (3.2 ± 1.3 mg/kg b.w., CyA trough levels 100–220 ng/ml), prednisone (7.5–10 mg daily), and azathioprine (100–150 mg daily). Time after renal transplantation ranged from 1.5–10 years. The control group comprised twelve gender- and age-matched healthy volunteers.

We evaluated thrombin activity (thrombin–antithrombin (TAT) complexes, Enzygnost TAT micro, Dade Behring, Germany; prothrombin fragments 1 + 2, Enzygnost F1 + 2 micro, Dade Behring) TAFI activator, thrombomodulin (TM) (IMUBIND thrombomodulin ELISA Kit, American Diagnostica, USA) – catalyser of TAFI activation, and the degree of plasmin generation (plasmin–antiplasmin (PAP) complexes, Enzygnost PAP micro, Dade Behring), with commercially available kits. We studied TAFI (TAFI-EIA, Affinity Biologicals, Canada) P-selectin (R&D Quantikine, USA), lipoprotein (a) (Lp(a), Biopool, Umeå, Sweden) with commercially available kits. All the tests were performed according to manufactures' instructions, by the same person. Total cholesterol, triglycerides, euglobulin clot lysis time, fibrinogen, and albumin concentration were measured by standard laboratory methods. To make euglobulin lysis time independent of fibrinogen concentration, we calculated the fibrinolytic activity index (FAI = fibrinogen divided by euglobulin lysis time). Statistical analysis was performed by means of ANOVA or Kruskal-Wallis ANOVA with post-hoc comparisons. *P* lower than 0.05 was considered significant.

Results

We found that fluvastatin treatment, with doses of 20 mg daily, taken at bedtime, safely and effectively reduced total cholesterol and LDL cholesterol in kidney transplant recipients. No adverse effects were observed. All the results are summarised in Table 1. The control group showed significantly lower serum lipid levels, Lp(a), TAFI, prothrombin fragments 1 + 2, TAT, TM, P-selectin and, significantly, FAI. In normolipaemic kidney transplant recipients, concentrations of creatinine, triglycerides, TAFI, F1 + 2, TAT and TM were significantly higher than those of healthy volunteers. Comparing two groups of kidney transplant recipients, we found that normolipaemic recipients had significantly lower serum lipids, TAFI, and FAI, than did hyperlipidaemic patients. In kidney transplant recipients, a significant drop in cholesterol, LDL, and triglycerides was found after 1, 2, and 3 months, whereas the HDL levels did not change significantly during 3 months of therapy with fluvastatin. Creatinine, serum albumin, Lp(a), TAT, PAP, and TM concentrations remained unchanged during fluvastatin treatment. The FAI increased significantly after 3 months of fluvastatin therapy, indicating an improvement in fibrinolysis, reaching levels observed in the control group. TAFI and prothrombin fragments 1 + 2 decreased significantly after 3 months of fluvastatin administration, reaching levels comparable to those of the control group. P-selectin decreased significantly after 2 months of fluvastatin therapy and remained significantly lower after 3 months of this therapy. Concentrations of P-selectin after 2 and 3 months of the treatment with fluvastatin did not differ significantly from those of the control group.

Discussion

As far as we know, this is the first report concerning effects of fluvastatin on TAFI concentration in a population of kidney transplant recipients. TAFI, a plasma zymogene when converted to an enzyme, potently inhibits fibrinolysis [5]. This indicates that increased levels of TAFI, reported by us previously [10] (over twice as high in transplant patients, than in healthy volunteers) and confirmed in this study, may be associated with an increased risk for thrombosis [11]. In their study, Van Tilburg et al. [11] found that persons whose TAFI levels exceeded the 90th percentile remained at increased risk for thrombosis. Our study [10, 12] and previous ones [13, 14] indicate that in kidney allograft

Table 1 Some biochemical and haemostatic parameters in kidney transplant recipients treated with fluvastatin (*ND* not done)

Parameter	Control group	Normolipaemic Tx	Before	After 1 month	After 2 months	After 3 months
Total cholesterol (mg/dl)	171.4 ± 45.2	190.0 ± 30.8††††	292.4 ± 36.7####	242.6 ± 25.9**##	231.8 ± 32.6***##	218.7 ± 21.5***#
LDL cholesterol (mg/dl)	110.6 ± 17.4	114.0 ± 35.2†††	215.9 ± 27.1####	170.2 ± 17.5**##	159.3 ± 20.4***##	149.2 ± 22.5***#
HDL cholesterol (mg/dl)	52.1 ± 8.2	51.8 ± 20.8	54.2 ± 13.1	53.3 ± 13.3	54.71 ± 12.7	56.7 ± 14.3
Triglycerides (mg/dl)	91.4 ± 12.9	143.1 ± 47.2†††#	172.7 ± 57.6####	155.4 ± 67.8##	145.8 ± 62.5*##	138.9 ± 70.2*##
Creatinine (mg/dl)	0.76 ± 0.18	1.74 ± 0.67##	1.52 ± 0.42##	1.58 ± 0.62##	1.60 ± 0.35##	1.46 ± 0.56##
Albumin (g/l)	4.59 ± 0.89	4.28 ± 0.51	4.29 ± 0.31	4.41 ± 0.49	4.32 ± 0.35	4.38 ± 0.27
TAFI (% of standard plasma)	114.2 ± 46.3	243.3 ± 115.3††	309.8 ± 239.9####	191.3 ± 96.3##	192.9 ± 129.4###	103.1 ± 55.6*
P-selectin (ng/ml)	69.67 ± 35.31	ND	114.52 ± 51.64#	103.68 ± 50.53	88.73 ± 27.31*	83.56 ± 12.87*
F 1 + 2 (nmol/l)	0.95 ± 0.71	2.09 ± 1.84##	1.90 ± 1.48##	1.26 ± 0.54#	1.46 ± 0.23#	1.08 ± 0.29*
FAI	1.48 ± 0.21	1.21 ± 0.35†	0.89 ± 0.32###	1.01 ± 0.27##	1.12 ± 0.28##	1.25 ± 0.39*
TM (ng/ml)	3.68 ± 1.53	7.93 ± 4.69###	8.19 ± 2.69###	7.69 ± 2.56###	7.93 ± 2.82####	7.22 ± 2.68####
TAT (μg/l)	1.09 ± 1.03	4.28 ± 3.64###	3.89 ± 2.99###	2.81 ± 1.79###	3.89 ± 1.94####	3.87 ± 2.95###
PAP (μg/l)	378.5 ± 113.8	515.8 ± 400.0	478.7 ± 131.6	378.7 ± 121.7	534.0 ± 88.1	432.8 ± 123.6
Lp (a) (mg/dl)	20.1 ± 4.9	ND	63.58 ± 25.86###	60.34 ± 30.12##	67.21 ± 20.31##	61.98 ± 18.52##

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, vs before therapy with fluvastatin
#$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, vs control group
†$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$, normolipaemic kidney transplant recipients vs before therapy with fluvastatin

recipients there is evidence of a higher degree of hypercoagulation as well as higher levels of atherogenic lipids. Since hypercoagulability has already been confirmed to be an independent risk factor for ischaemic heart disease, several studies have implicated thrombosis as a complication of kidney transplantation [14]. Therefore, a decrease in TAFI by 3 months of treatment of hyperlipidaemia with fluvastatin may suggest an improvement in fibrinolysis in these patients. It is unknown at present whether the observed phenomenon is clinically relevant. To the authors' knowledge, there are no data on correlations between elevated TAFI levels and thrombo-embolic complications in kidney transplant recipients. In our previous study we reported a significant decrease in TAFI and TM concentrations in hyperlipidaemic CAPD patients after 6 months of treatment with simvastatin [15], without any significant changes in fibrinogen, ECLT and markers of ongoing coagulation and fibrinolysis, TAT and PAP complexes. As shown by van Tilburg et al. [11], most persons with elevated TAFI antigen exceeding the 90th percentile (over 122 U/dl) also had elevated plasma levels of some other factors (fibrinogen, antithrombin III, prothrombin, protein C). However, none of these was responsible for the risk for thrombosis associated with elevated TAFI levels. In our study we assessed only fibrinogen and found no changes in its concentration during fluvastatin therapy.

Prothrombin fragments 1 + 2, a marker of thrombin generation, is strongly associated with an increased risk of cardiovascular events and adverse clinical outcome [16]. We found a significant decrease in F1 + 2 after 3 months of fluvastatin therapy, in kidney transplant recipients, similar to the results obtained by Joukhadar et al. [17], who reported a significant decrease in F1 + 2 in hypercholesterolaemic patients following 3 months of simvastatin administration. We did not observe any changes in TAT complexes, a marker of intravascular thrombin generation, or in PAP complexes, a marker of the activity status of the fibrinolytic system. This was probably due to a wide range of TAT and PAP values. We measured ECLT, which reflects overall fibrinolytic activity. It depends also on the balance between plasminogen activators and inhibitors. To make it independent of fibrinogen, we calculated the FAI. This increased after 3 months of fluvastatin therapy, indicating improvement in fibrinolysis, usually impaired in kidney transplant recipients [18]. Another interesting finding in our study was a significant decrease in P-selectin concentration after 2 and 3 months of fluvastatin treatment. Soluble P-selectin has been hypothesised to play a role in the initiation of atherosclerosis [19], a common finding in kidney transplant recipients. On the other hand, P-selectin, though it may be derived from either platelets and/or endothelium [20], has potential as a new marker of platelet activation [21]. Platelet activity

is enhanced in hypercholesterolaemia and may be a crucial factor in the pathogenesis of atherosclerotic lesion formation, and at least partially in the occurrence of cardiovascular events [22]. Activated platelets tend to aggregate and are found close to atherosclerotic plaques [23]. As we have shown previously [24], platelet hyperactivity and hypercholesterolaemia are common findings in kidney transplant recipients. This remains a common problem following successful engraftment. It has been reported that fluvastatin and other statins reduce platelet activity in hypercholesterolaemia [22, 25], therefore, a significant decrease in P-selectin following fluvastatin treatment may suggest a decrease in platelet hyperactivity and/or amelioration in endothelial dysfunction. Ambrosi et al. [8] reported a decrease in soluble TM in cardiac transplant recipients after 4 weeks of fluvastatin therapy. In our study we did not observe such a significant change in TM concentration in kidney transplant recipients following 3 months of fluvastatin administration. It could be due to the fact that we administered 20 mg of fluvastatin, instead of 40 mg as in the study of Ambrosi et al. [8]. However, we performed a longitudinal study, not a crossover one, as they did. [8], Furthermore, we assessed TM concentration using a commercially available kit from American Diagnostica, USA, not with one from Diagnostica Stago, France, and we obtained levels that were 9–10 times lower than those obtained by Ambrosi et al. [8]. In our study, all the patients were on triple immunosuppressive regimen comprising CyA, azathioprine, and prednisone, whereas in the previously cited study [8] all the cardiac transplant recipients received CyA; some of them were also given azathioprine (12 of 20), and 8 of 20 were administered prednisone. Ambrosi et al. do not state at which doses these drugs were administered.

Patients were enrolled in this study on the basis of having cholesterol levels over 240 mg%. We chose fluvastatin bearing in mind that the use of other HMG-CoA reductase inhibitors had been limited in the past, due to potentially serious drug interactions with cyclosporine. In our patients, mean CyA levels remained constant throughout the study, and adverse effects were not observed. We found a significant reduction in cholesterol, LDL, and triglyceride levels without any significant changes in HDL, in kidney allograft recipients following short-term fluvastatin administration. Similar data were published by Goldberg and Roth [26], but they did not find any changes in triglyceride level after 14 weeks of fluvastatin administration. Lp(a), a cholesterol-carrying lipoprotein that is like LDL, is reported to be an independent risk factor for coronary heart disease [27] and is increased in kidney allograft recipients [12]. Its homology with plasminogen may predispose to thrombosis. In our study, Lp(a) concentrations were not influenced by short-term fluvastatin therapy. It has been observed that both simvastatin and lovastatin also did not alter Lp(a) levels in kidney allograft recipients [28].

We conclude that our study has theoretical and practical implications, since fluvastatin effects on TAFI, prothrombin fragments 1 + 2, fibrinolytic activity, and P-selectin in kidney transplant recipients, might, besides lowering cholesterol and triglycerides, improve atherogenic and thrombogenic tendencies in this population. Whether these changes are of clinical significance (reduction in the morbidity and mortality of vascular disease) remains to be investigated.

References

1. Locatelli F, Del Vecchio L, Manzoni C (1998) Morbidity and mortality on maintenance hemodialysis. Nephron 80:380–400
2. US Renal Data System (1999) USRDS 1999 Annual Data Report. National Institute of Health, National Institute of Diabetes and Digestive and Kidney Disease, Bethesda, Md
3. Pirsch JD, D'Alessandro AM, Solinger HW, Knechtle SJ, Reed A, Klayoglu M, Belzer FO (1992) Hyperlipidemia and transplantation: etiologic factors and therapy. J Am Soc Nephrol 2:238–242
4. Sweny P, Wheeler DC, Lui SF, Amin NS, Barradas MA, Jeremy JY, Mikhailidis DP, Varghese Z, Fernando ON, Moorhead JF (1989) Dietary fish oil supplements preserve renal function in renal transplant recipients with chronic vascular rejection. Nephrol Dial Transplant 4:1070–1075
5. Nesheim M, Wang W, Boffa M, Nagashima M, Morser J, Bajzar L (1997) Thrombin, thrombomodulin and TAFI in the molecular link between coagulation and fibrinolysis. Thromb Haemost 78:386–391
6. Wang W, Boffa MB, Bajzar L, Walker JB, Nesheim ME (1998) A study of the mechanism of inhibition of fibrinolysis by activated thrombin-activatable fibrinolysis inhibitor. J Biol Chem 273:27176–27181
7. Bajzar L, Nesheim ME, Morser J, Tracy PB (1998) Both cellular and soluble forms of thrombomodulin inhibit fibrinolysis by potentiating the activation of thrombin-activatable fibrinolysis inhibitor. J Biol Chem 273:2793–2798
8. Ambrosi P, Ailaud MF, Habib G, Kreitman B, Metras D, Luccioni R, Bouvenot G, Juhan-Vague I (2000) Fluvastatin decreases soluble thrombomodulin in cardiac transplant recipients. Thromb Haemost 83:46–48
9. Malyszko J, Urano T, Knofler R, Taminato A, Yoshimi T, Takada Y, Takada A (1994) Daily variations of platelet aggregation in relation to blood and plasma serotonin in diabetes. Thromb Res 75:569–576

10. Hryszko T, Malyszko J, Malyszko JS, Brzosko S, Pawlak K, Mysliwiec M (2001) A possible role of thrombin-activatable fibrinolysis inhibitor in disturbances of fibrinolytic system in renal transplant recipients. Nephrol Dial Transplant 16:1692–1696
11. van Tilburg NH, Rosendaal FR, Bertina RM (2000) Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. Blood 95:2855–2859
12. Malyszko J, Malyszko JS, Pawlak K, Mysliwiec M (1996) Coagulo-lytic system and endothelial function in cyclosporine-treated kidney allograft recipients. Transplantation 62:828–830
13. Baker LRI, Tucker B, Kovacs IB (1990) Enhanced in vitro hemostasis and reduced thrombolysis in cyclosporine-treated renal transplant recipients. Transplantation 49:905–909
14. Venrenterghem Y, Roels I, Lerut T, Gruwez J, Michielsen P, Gresele P, Deckmyn H, Colucci M, Arnout J, Vermylen J (1985) Thromboembolic complications and haemostatic changes in cyclosporin-treated cadaver kidney allograft recipients. Lancet:999–1002
15. Malyszko J, Malyszko JS, Hryszko T, Mysliwiec M (2001) Simvastatin affects TAFI and thrombomodulin in CAPD patients. Thromb Haemost 86:900–901
16. Agewall S, Wikstrand J, Fagerberg B (1998) Prothrombin fragment 1+2 is a risk factor for myocardial infarction in treated hypertensive men. J Hypertens 16:537–541
17. Joukhadar C, Klein N, Prinz M, Schrolnberger C, Vukovich T, Woltz M, Schmetter L, Dorner GT (2001) Similar effects of atorvastatin, simvastatin and pravastatin on thrombogenic and inflammatory parameters in patients with hypercholesterolemia. Thromb Haemost 85:47–51
18. Patrassi GM, Sartori MT, Rigotti P, Di Landro D, Theodoridis P, Fioretti M, Capalbo M, Saggiorato G, Boeri G, Girolami A (1995) Reduced fibrinolytic potential one year after kidney transplantation. Relationship to long-term steroid treatment. Transplantation 59:1416–1420
19. Ridker PM, Buring JE, Rifai N (2001) Soluble P-selectin and the risk of future cardiovascular events. Circulation 103:491–495
20. Johnston GI, Bliss GA, Newman PJ, McEver RP (1990) Structure of the human gene encoding granule membrane protein-140, a member of the selectin family of adhesion receptors for leukocytes. J Biol Chem 265:21381–21385
21. Blann AD, Lip GYH (1997) Hypothesis: is soluble P-selectin a new marker of platelet activation? Atherosclerosis 128:135–138
22. Aviram M, Hussein O, Rosenblat M, Schlezinger S, Hayek T, Keidar S (1998) Interactions of platelets, macrophages and lipoproteins in hypercholesterolemia: antiatherogenic effects of HMG-CoA reductase inhibitor therapy. J Cardiovasc Pharmacol 31:39–45
23. Schror K (1990) Platelet reactivity and arachidonic acid metabolism in type II hyperlipoproteinemia and its modification by cholesterol lowering agents. Eicosanoids 3:67–73
24. Malyszko J, Malyszko JS, Pawlak D, Pawlak K, Buczko W, Mysliwiec M (1996) Platelet aggregation and peripheral serotonergic system in kidney transplant recipients treated with cyclosporin A. Transplant Proc 28:1954–1957
25. Huhle G, Abletshauser C, Mayer N, Weidinger G, Harenberg J, Heene DL (1999) Reduction of platelet activity markers in type II hypercholesterolemic patients by a HMG-CoA reductase inhibitor. Thromb Res 95:229–234
26. Goldberg RB, Roth D (1995) A preliminary report of the safety and efficacy of fluvastatin for hypercholesterolemia in renal transplant patients receiving cyclosporine. Am J Cardiol 76:107A–109A
27. Loscalzo J (1990) Lipoprotein (a). A unique risk factor for atherothrombotic disease. Arteriosclerosis. 10:672–680
28. Gault MH, Longerich LL, Purchase L, Harnett J, Breckenridge C (1995) Comparison of Lp(a) concentrations and some potential effects in hemodialysis, CAPD, transplantation, and control groups: a review of the literature. Nephron 70:155–165