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Effects of leflunomide therapy on serum lipid and lipoprotein profiles in patients with rheumatoid arthritis (RA)

Cardiovascular morbidity and mortality is increased in patients with rheumatoid arthritis (RA), which might be due to an increased prevalence of cardiovascular risk factors such as dyslipidemia and dyslipoproteinemia (10, 14). The cause of this enhanced cardiovascular risk is unknown, but inflammation is thought to play an important part (4) which is in line with the accumulating evidence that inflammation has a prominent role in the development of atherosclerosis (9, 12).

Our aim was to investigate the effects of leflunomide therapy on lipid and apolipoprotein (apo)AI, apoB, apoCIII, apoCIIInonB, apoE, apoEnonB and apoB-containing apoCIII and apoE in patients with rheumatoid arthritis.

MATERIAL AND METHODS

The study group consisted of 10 patients (age 27–78 years) with RA, treated with leflunomide dose of 20mg/day. The serum levels of lipid and apolipoprotein (apo)AI, apoB, apoCIII, apoCIIInonB, apoE, apoEnonB and apoB-containing apoCIII and apoE were determined at baseline and after 3 and 6 months of treatment. In our group 3 patients had hypertension, 1 patient had diabetes and 1patients had glucose intolerance. In the treatment we included also the following medicaments 8 patients got low dose corticosteroids, 3 patients got angiotensin converting enzyme inhibitor (ACEI), 7 patients received steroid anti-inflammatory drugs (NSAID), 2 patients received analgesics drugs and 1patients was treated with insulin.

Lipid and lipoprotein profiles were obtained in serum after 14-hour overnight fasting. Lipids and lipoproteins were determined on Hitachi 902 analyser. The total cholesterol (TC) was estimated by the enzymatic-colorimetric method, BIOMAXIMA, HDL-C by the direct method with immunoinhibition (AB-VACO - BIOMAXIMA). HDL cholesterol, which is not bound with enzymes (cholesterol esterase and cholesterol oxidase) and chromogens producing a coloured complex were determined. Triglycerides (TG) were determined using the standard enzymatic technique (BIOMAXIMA). LDL-cholesterol (LDL-C) was calculated according to the Friedewald formula. Non-HDL-C was calculated as total cholesterol minus HDL-C. Apo AI, apoB were measured by Roche kit using the turbidimetric methods. TRL were separated as non-HDL lipoproteins from apoCIIInonB in the HDL fraction using anti-apoB antibodies. The method with applied anti-apoB antibody separating apoB-containing lipoproteins in VLDL+LDL (non-HDL) and non-apoB-containing lipoproteins in HDL can be used in diagnosis and treatment of patients both with chronic allograft nephropathy and atherosclerosis in post renal transplant patients. Lipoproteins (total apoCIII, apoCIIInonB and

total apoE and apoEnonB) were measured by electroimmunodiffusion according to Laurell using a commercial kit Sebia, USA.

RESULTS

Total cholesterol (TC), triglicerides (TG), LDL-C, and non-HDL-C were significantly increased as compared to reference group and not significantly increased after 3 and 6 months treatment. ApoB concentration at baseline and after 3 and 6 months were not changed. The concentration of HDL-C well as apoAI were improve after 3 and 6 months. Moreover, total apoE and apoEnonB were not significantly increased as compared to reference group at baseline and increased after 3 months and 6 months, respectively but apoB:E were not changed. The concentration of total apo CIII, apoCIIInonB, and apoB:CIII at baseline and 3 and 6 months were not changed. The atherogenic lipid and lipoprotein ratios at baseline were more favorable after 3 and 6 months treatment.

Table 1. Lipid and lipoprotein profiles in reference group and in patients with R A at baseline and after 3- and 6-months treatment with leflunomide

	Reference group n= 32	Basal n = 10	After 3month n= 10	After 6 month n = 10
TG	97.7 ± 16.3	132.11 ± 46.6[a]	132.5 ± 74.6[a]	131.5 ± 73.2[a]
TC	180.9 ± 60.0	223.8 ± 37.6[a]	215.6 ± 40.5[a]	233.3 ± 42.8[a]
LDL-C	103.9 ± 42.6	148.6 ± 34.9[a]	144.8 ± 36.9[a]	153.6 ± 39.8[a]
HDL-C	60.3 ± 12.7	45.1 ± 8.2[a]	46.8 ± 15.6[a]	52.0 ± 15.6
Non-HDL-C	122.3 ± 17.2	174.8 ± 40.7[a]	166.8 ± 44.8[a]	178.5 ± 48.1[a]
apoAI	167.6 ± 16.7	170.5 ± 34.9	167.6 ± 34.9	178.1 ± 32.7
apoB	90.2 ± 14.8	111.3 ± 27.8	95.5 ± 19.4	109.7 ± 32.2
apoE	5.18 ± .,92	5.13 ± 1.15	5.90 ± 1.07	6.9 ± 2.24[a]
apoEnonB	4.21 ± 1.08	4.45 ± 1.03	5.24 ± 1.15	5.98 ± 1.65[a]
apoB: E	0.98 ± 0.78	0.68 ± 0.53	0.76 ± 0.46	0.92 ± 0.72
apoCIII	2.64 ± 0.51	2.87 ± 0.89	2.98 ± 1.14	3.01 ± 1.19
apoCIIInonB	1.86 ± 0.41	2.07 ± 0.45	2.04 ± 0.55	2.26 ± 0.85
apoB: CIII	0.78 ± 0.23	0.805 ± 0.53	0.985 ± 0.79	0.74 ± 0.69
TC/HDL-C	3.40 ± 0.75	5.08 ± 1.58[a]	4.88 ± 1.81[a]	4.57 ± 1.55[a]
LDL-C/HDL-C	2.15 ± 0.68	3.53 ± 1.29[a]	3.22 ± 1.42[a]	3.09 ± 1.24[a]
TG/HDL-C	1.68 0.13	3.05 ± 1.35[a]	3.27 ± 2.59[a]	2.73 ± 1.95
apoAI/apoB	1.80 ± 0.40	1.65 ± 0.53	1.82 ± 0.55	1.75 ± 0.61
apoAI/apoCIII	57.85 ± 9.3	59.27 ± 11.2	57.00 ± 12.1	59.13 ± 11.8

a – vs. reference group

DISCUSSION

Our investigation of patients with RA shows that disturbed lipid and lipoprotein profiles caused on acceleration of atherosclerosis and cardiovascular disease (10, 14). It was suggested that inflammation can also increased the cardiovascular risk by deterioration of the lipid profile (9). Other investigators found an association between increased lipids such as oxidised low-density lipoprotein cholesterol and proinflammatory cytokines such as CRP, interleukin 6 and tumor necrosis factor (7, 11). The findings of the present study confirm these effects of inflammation on the various lipid concentrations. This is supported by the demonstration of a decrease in HDL-C and apoAI levels

and an increase in triglycerides and apoB levels during an acute-phase response (2, 6). We found higher concentration of lipids (especially TC, LDL-C and non-HDL-C before treatment but after 3 and 6 of months concentration of these lipids was not changed). The concentration of HDL-C was decreased before and increased after 3 and 6 months of treatment of leflunomide. Corticosteroid use at baseline and 3 and 6 months treatment was associated with higher HDL-C and apoAI levels. Corticosteroids may raise HDL-C levels by increasing apoA-I production by the liver or/and via a mechanism decreasing CETP activity. Significantly reduced HDL-C levels and higher plasma CETP activity were observed after prednisone therapy (5).

Several reports show anti-inflammatory effects of HDL and particularly apoAI. It was suggested that apoAI is able to inhibit interactions between T lymphocytes and monocytes, thereby modulating the inflammatory response (1). Moreover, another study showed the ability of apoAI to inhibit interleukin 1 and tumor necrosis factor alfa, which further supports the theory of an active modulating role of lipids in inflammation (8).

Apolipoprotein C (apoC) is associated with chylomicrons, VLDL-C a more favorable ApoE and apoCIII concentrations in VLDL and LDL are associated with coronary heart disease. ApoCIII modulates the metabolism of VLDL by inhibiting lipoprotein lipase activation by apoCII, and the binding of apoE to hepatic lipoprotein receptors. ApoCIII can also inhibit hepatic lipase, which plays an important role in the conversion of dense VLDL to IDL and LDL. Hipertriglyceridemic patients have increased plasma apoCIII concentrations and production rates compared with normolipidemic. In contrast, apoCIII – deficient plasma have reduced VLDL triglyceride with rapid conversion of VLDL to LDL. It has been suggested that apoB lipoproteins (VLDL, IDL, LDL) that have apoCIII are atherogenic in humans (3). ApoCIII concentrations in plasma and in apoB lipoproteins are higher among patients with coronary disease than among controls. The interpretation of the role of apoCIII is complicated by the presence of apoE in VLDL in addition to apoCIII. ApoE can be recognized by several cell surface receptors, facilitating the clearance of VLDL. Paradoxically, apoE concentrations are high in hypertriglyceridemic patients and are associated with coronary heart disease (3, 13). ApoCIII interferes with the function of apoE to bind cell surface LDL and LDL receptor-related protein receptors. Triglyceride-rich particles without apoCIII or apoE could be the most important precursors of plasma LDL, and this lipolytic pathway could play an important role in hypercholesterolemic patients, who have high concentrations of these particles (13).

Our studies suggest that treatment with leflunomide made lipid and lipoprotein profiles more favorable which was marked by lipid and lipoprotein ratios.

CONCLUSION

The study presented indicate that the patients with rheumatoid arthritis show an athrogenic lipid and lipoprotein profiles and that treatment with leflunomide make lipid and lipoprotein profiles and atherogenic indexes more favorable although long term investigations are needed to confirm this.

REFERENCES

1. Burger D., Dayer J. M.: High-density lipoprotein-associated withapolipoprotein A-I: the missing link between infection and chronic inflammation? Autoimmun. Rev., 1, 111, 2002.
2. Cabana V. G., Siegel J. N., Sabesin S. M.: Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins. J. Lipid Res., 30, 39, 1989.

3. Campos H., Perlov D., Khoo C. et al.: Distinct patterns of lipoproteins with apoB defined by presence of apoE or apoCIII in hypercholesterolemia and hypertriglyceridemia. J. Lipid Res., 42, 1239, 2001.
4. Choi H. K., Hernan M. A., Seeger J. D., Robins J. M., Wolfe F.: Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. Lancet, 359, 1173, 2002.
5. Donahoo W. T., Kosmiski L. A., Eckel R. H.: Drugs causing dyslipoproteinemia. Endocrinol. Metab. Clin. North. Am., 27, 677, 1998.
6. Halm V. P, Nielen M. M. L., Nurmohamed M. T., Schaardenburg D., Reesink H. W., Voskuyl A. E. et al.: Lipids and inflammation: serial measurements of the lipid profile of blood donors who later developed rheumatoid arthritis. Ann. Rheum. Dis., 66, 184, 2007.
7. Hulthe J., Fagerberg B.: Circulating oxidised LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). Atheroscler. Thromb. Vasc. Biol., 22, 1162, 2002.
8. Hyka N., Dayer J. M., Modoux C., Kohno T., Edwards C. K. III, Roux-Lombard P. et al.: Apolipopprotein AI inhibits the production of interleukin -1 beta and tumor necrosis factor-alpha by bloking contact-mediated activation of monocytes by T lymphocytes. Blood, 97, 2381, 2001.
9. Libby P.: Inflammation in atherosclerosis. Nature, 420, 868, 2002.
10. Lindqvist E., Eberhardt K.: Mortality in rheumatoid arthritis patients with disease onset in the 1980s. Ann. Rheum. Dis., 58, 11, 1999.
11. Menotti A., Lanti M.: Coronary risk factors predicting early and late coronary deaths. Heart, 89, 19, 2003.
12. Ross R.: Atherosclerosis – an inlfammatory disease. N. Engl. J. Med., 340, 115, 1999.
13. Sacks F. M., Alaupovic P., Lemuel A. et al.: VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the cholesterol and recurrent events (CARE) trial. Circulation, 102, 1886, 2000.
14. Solomon D. H., Karlson E. W., Rimm E. B., Cannusio C. C., Mandl L.A., Manson J. E. et al.: Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis. Circulation, 107, 1303, 2003.

SUMMARY

The study group consisted of 10 patients (27-78 years) with RA, treated with leflunomide 20mg/day. The reference group was made up of 13 healthy subjects (22-57 years). The serum levels of lipids and apoAI, apoB, apoCIII, apoCIIInonB, apoE, apoEnonB and apoB-containing apoCIII (apoB:CIII) and apoE apoEnonB, apoB:E were determined at baseline and after 3 and 6 months of treatment. Lipid and lipoprotein profiles were obtained in serum after 14-hour overnight fasting. Total cholesterol (TC), LDL-C, triglicerydes (TG) and non-HDL-C were significantly increased when compared to the reference group and not significantly increased at 3 and 6 months of treatment. ApoB concentration at baseline and at 3 and 6 months were not changed. The concentrations of HDL-C, apoAI, the atherogenic lipid and lipoprotein ratios were improved at 3 and 6 months. Moreover, the total apoE and apoEnonB were not significantly increased as compared to the reference group at baseline and increased at 3 months and 6 months, respectively, but apoB:E were not changed. The concentrations of trigliceride and total apo CIII, apoCIIInonB, and apoB:CIII concentration at baseline and after 3 and 6 months were not changed. at baseline were more favorable after 3 and 6 months of treatment. The study presented indicate that the patients with RA show athrogenic lipid

and lipoprotein profiles but the treatment with leflunomide makes lipid and lipoprotein profiles and atherogenic indexes more favorable, although long term investigations are needed to confirm this.

Wpływ leczenia leflunomidem na profil lipidowy i lipoproteinowy pacjentów z reumatoidalnym zapaleniem stawów (RA)

Badana grupa stanowiła 10 pacjentów (27–78 lat) z RA, leczonych leflunomidem 20 mg/dobę. Grupa referencyjna obejmowała 13 zdrowych osób (22–57 lat). W surowicy pacjentów oznaczono stężenia lipidów i apoAI, apoB, apoCIII, apoCIIInieB, apoB:CIII, apoE, apoEnieB, apoB:E przed oraz w 3 i 6 miesiącu leczenia leflunomidem. W badaniach wykazano istotny wzrost stężenia TG, TC, LDL-C i cholesterol zawarty poza frakcją HDL (non-HDL-C) w porównaniu z grupą referencyjną, co nie uległo istotnym zmianom po leczeniu. Stężenia HDL-C, apoAI, wartości wskaźników lipidowych i lipoproteinowych wykazywały korzystniejsze zmiany w 3 i 6 miesiącu leczenia. Stężenia apoE, apoEnieB i apoB:E nie były istotnie podwyższone przed leczeniem w porównaniu z grupą referencyjną i wzrosły w 3 i 6 miesiącu leczenia. Natomiast stężenia apoCIII, apoCIIInieB i apoB:CIII nie były istotnie podwyższone w porównaniu z grupą referencyjną przed leczeniem i nie wykazywały znamiennego wzrostu w 3 i 6 miesiącu leczenia. Przeprowadzone badania prezentują miażdżycowy profil lipidowy i lipoproteinowy pacjentów z RA, a leczenie leflunomidem nie pogłębia tych zaburzeń, ale wykazuje korzystniejsze tendencje, jednak dalsze badania w tym kierunku są wymagane.