

Fatty Acid Composition of Plasma and Kidney in Rats with Anti-Thy1.1 Nephritis

RYUICHIRO KUMASAKA¹, NORIO NAKAMURA¹, HIDEAKI YAMABE¹, HIROSHI OSAWA¹,
KEN-ICHI SHIRATO¹, MICHIKO SHIMADA¹, REIICHI MURAKAMI¹,
TAKESHI FUJITA¹, KEN OKUMURA¹, KEI HAMAZAKI² and TOMOHITO HAMAZAKI²

¹Department of Nephrology, Hirosaki University School of Medicine, Aomori;

²Department of Clinical Sciences, Institute of Natural Medicine, University of Toyama, Toyama, Japan

Abstract. *Background:* n-3 Polyunsaturated fatty acids (PUFA) are reported to ameliorate atherosclerotic and inflammatory diseases because they compete with arachidonic acid and reduce its inflammatory metabolites. In the present study, the fatty acid composition of plasma and kidney in rats with anti-Thy1.1 nephritis was investigated. *Materials and Methods:* A group of male Wistar rats weighing about 200 g was injected with anti-Thy1.1 antibody (1.25 mL/kg) through their tail veins (nephritis group). Rats in the control group were injected with saline. Five days after the injection, urinary protein levels were determined. All rats were then sacrificed and fatty acid composition of plasma and kidney were analyzed. *Results:* Eicosapentaenoic acids (EPA) levels in the kidney phospholipid (PL) fraction in the nephritis group were significantly lower than those in the control group (0.67 ± 0.06 mol% vs. 0.96 ± 0.06 mol%, $p < 0.05$). EPA levels in the plasma PL fraction in the nephritis group were also significantly lower than those in the control group (0.38 ± 0.05 mol% vs. 0.59 ± 0.03 mol%, $p < 0.05$). Urinary protein levels 5 days after the injection were inversely correlated with EPA levels in the kidney PL fraction ($r^2 = 0.65$, $p = 0.01$). These results suggested that decreased EPA levels in the kidney PL fraction might play an important role in anti-Thy1.1 nephritis.

n-3 Polyunsaturated fatty acids (PUFA) are reported to ameliorate atherosclerotic and inflammatory diseases, because they compete with arachidonic acid and reduce its inflammatory metabolites, namely prostaglandins and leukotrienes (1).

Correspondence to: Norio Nakamura, MD, Department of Nephrology, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki-city, Aomori, 036-8562, Japan. Tel: +81 172 39 5057, Fax: +81 172 35 9190, e-mail: nnakamur@r2.dion.ne.jp

Key Words: Eicosapentaenoic acid (EPA), fatty acid composition, polyunsaturated fatty acid (PUFA), Thy1.1 nephritis.

Anti-Thy1.1 nephritis is characterized by acute complement-mediated mesangiolysis, followed by mesangial hypercellularity and extracellular matrix accumulation (2). This model is now widely used as a model of mesangial proliferative glomerulonephritis.

There were several reports about the beneficial effects of n-3 PUFA on mesangial proliferative glomerulonephritis such as IgA nephropathy (3, 4). However, there are no reports about the metabolism of PUFA in mesangial proliferative glomerulonephritis.

In the present study, the fatty acid composition of plasma and kidney in rats with anti-Thy1.1 nephritis as mesangial proliferative nephritis was investigated.

Materials and Methods

Eight male Wistar rats weighing about 200 g were used in the present study. Three were injected with anti-Thy1.1 antibody (1.25 mL/kg, COSMO BIO Co. Ltd., Tokyo, Japan) through their tail veins (nephritis group). The other 5 rats were injected with saline (control group). Five days after the injection, urinary protein levels were determined. All rats were then sacrificed, and their plasma and kidneys were obtained. Total protein levels, total cholesterol levels and creatinine levels in plasma were analyzed enzymatically. Urinary protein levels were also analyzed enzymatically. The fatty acid composition of plasma and kidney was analyzed as follows: total lipids were extracted by the methods of Folch *et al.* (5) and were separated into phospholipids (PL) and other fractions by thin-layer chromatography. Fatty acids in the PL fraction were transmethylated with 6% sulfuric acid in anhydrous methanol and analyzed on a C-14A gas-chromatograph (Shimazu, Kyoto, Japan) equipped with an SP-2330 capillary column (Supelco, Bellefonte, PA, USA) (6).

All data were expressed as means \pm SE. Statistical analysis was performed using an unpaired *t*-test. A *p* value < 0.05 was considered statistically significant.

Results

The fatty acid composition in the kidney PL fraction is shown in Table I. 18:2n-6, 18:3n-6 and eicosapentaenoic

Table I. Fatty acid composition in the phospholipid fraction of the kidney.

Fatty acid	Control	Thy1.1 nephritis
16:0	22.06±0.64	22.39±0.55
18:0	18.07±0.43	18.73±0.22
18:1n-9	6.18±0.13	6.58±0.41
18:2n-6	12.79±0.25	11.87±0.10*
18:3n-6	0.12±0.02	N.D.*
18:3n-3	0.09±0.03	0.02±0.02
20:3n-6	1.40±0.09	1.14±0.18
20:4n-6	26.85±0.29	26.76±0.40
20:5n-3	0.96±0.06	0.67±0.06*
22:5n-3	0.44±0.02	0.52±0.09
22:6n-3	3.04±0.13	3.29±0.02
EPA/AA	0.034±0.002	0.027±0.003
n-6/n-3	9.204±0.332	8.973±0.124
AA/PUFA	0.580±0.003	0.590±0.006

Data are expressed as means±S.E. *p<0.05. AA: Arachidonic acid (20:4n-6), EPA: eicosapentaenoic acid (20:5n-3); PUFA: polyunsaturated fatty acid. n-6/n-3=(18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6)/(18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3); AA/PUFA=20:4n-6/(18:2n-6 + 18:3n-6 + 18:3n-3 + 20:3n-6 + 20:4n-6 + 20:5n-3 + 22:5n-3 + 22:6n-3).

acid (EPA; 20:5n-3) levels in the nephritis group were significantly lower than those in the control group.

The fatty acid composition in the plasma PL fraction is shown in Table II. 18:2n-6 and EPA levels in the nephritis group were significantly lower than those in the control group. Docosahexaenoic acid (DHA; 22:6n-3) levels in the nephritis group were significantly higher than those in the control group. The ratios of EPA to AA and n-6 PUFA to n-3 PUFA in the nephritis group were significantly lower than those in the control group.

Total protein, total cholesterol and creatinine levels in plasma are shown in Table III. Total protein levels in the nephritis group were significantly lower than those in the control group. Total cholesterol levels in the nephritis group were significantly higher than those in the control group. There were no significant differences in plasma creatinine levels between the nephritis and control groups.

Urinary protein levels 5 days after the injection of anti-Thy1.1 antibody in the nephritis group were significantly higher than those in the control group (Table IV). Urinary protein levels 5 days after the injection were inversely correlated with EPA levels in the kidney PL fraction ($r^2=0.65$, $p=0.01$).

Discussion

In the present study, we found that EPA levels in the kidney and plasma PL fraction in rats with anti-Thy1.1 nephritis were significantly lower than those in the control group.

The mechanism for the EPA levels in the kidney and plasma PL fraction in rats with anti-Thy1.1 nephritis being

Table II. Fatty acid composition in the phospholipid fraction of the plasma.

Fatty acid	Control	Thy1.1 nephritis
16:0	21.50±0.23	22.07±0.38
18:0	21.56±0.33	22.36±0.79
18:1n-9	4.79±0.28	4.15±0.41
18:2n-6	21.65±0.30	19.28±0.34**
18:3n-6	0.13±0.06	0.12±0.06
18:3n-3	0.75±0.40	0.49±0.18
20:3n-6	1.30±0.19	0.93±0.16
20:4n-6	16.50±0.72	18.04±0.29
20:5n-3	0.59±0.03	0.38±0.05*
22:5n-3	0.47±0.09	0.67±0.08
22:6n-3	4.35±0.17	6.48±0.85*
EPA/AA	0.035±0.003	0.023±0.003*
n-6/n-3	6.488±0.337	4.917±0.447*
AA/PUFA	0.355±0.013	0.383±0.003

Data are expressed as means±S.E. *p<0.05, **p<0.01. AA: Arachidonic acid (20:4n-6); EPA: eicosapentaenoic acid (20:5n-3); PUFA: polyunsaturated fatty acid. n-6/n-3=(18:2n-6 + 18:3n-6 + 20:3n-6 + 20:4n-6)/(18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3); AA/PUFA=20:4n-6/(18:2n-6 + 18:3n-6 + 18:3n-3 + 20:3n-6 + 20:4n-6 + 20:5n-3 + 22:5n-3 + 22:6n-3).

Table III. Total protein, total cholesterol and creatinine concentrations in the plasma (mg/dL).

	Control	Thy1.1 nephritis
Total protein	6.14±0.08	5.77±0.15*
Total cholesterol	73.60±3.54	125.67±23.54*
Creatinine	0.22±0.01	0.42±0.16

Data are expressed as means±S.E. *p<0.05.

Table IV. Urinary protein levels at 5 days after the injection of Thy1.1 antibody.

	Control	Thy1.1 nephritis
Urinary protein (mg/day)	10.12±0.45	70.96±2.44*

Data are expressed as means±S.E. *p<0.001.

significantly reduced was not clear, but the following mechanism might explain the results: a) EPA in the kidney PL fraction is consumed by the inflammation in anti-Thy1.1 nephritis; b) the conversion of EPA to DHA is increased in anti-Thy1.1 nephritis. The activities of desaturase and elongase might be increased in the nephritis. However, in the kidney PL fraction, DHA levels in Thy1.1 nephritis were not higher than those in the control group. These results were incompatible with our speculation.

Prostaglandin I₃ from EPA has inhibitory effects on platelet aggregation and vaso-constriction, and leukotriene B₅ from EPA has much less effects on activating leukocytes than leukotriene B₄ from arachidonic acids (AA) (7). Consequently, decreased EPA levels in the kidney and plasma PL fractions and decreased EPA/AA in the plasma PL fraction might cause the progression of nephritis. Moreover, urinary protein levels 5 days after the injection were inversely correlated with EPA levels in the kidney PL fraction. These results suggested that decreased EPA might play an important role in Thy1.1 nephritis.

In the present study, the levels of 18:2n-6 and 18:3n-6 in the kidney and plasma PL fractions in Thy1.1 nephritis were lower than those in the control group. The mechanism is not clear, but the desaturases that produce PUFA are expressed in the liver, heart, and brain and are regulated by hormonal and nutritional manipulation (8). We also reported that fatty acid metabolism was altered in patients with nephrotic syndrome (9). Consequently, it is possible that fatty acid metabolism might be altered in Thy1.1 nephritis.

The ratios of n-6 PUFA to n-3 PUFA in Thy1.1 nephritis were significantly lower than those in the control rats in the plasma PL fraction, probably because DHA levels in Thy1.1 nephritis were higher than those in the control group.

Conclusion

We showed that EPA levels in the kidney and plasma PL fractions in rats with anti-Thy1.1 nephritis were significantly lower than those in the control rats. These results suggest that decreased EPA levels in the kidney and plasma PL fractions might play an important role in anti-Thy1.1 nephritis.

Acknowledgements

This study was supported in part by grant from The Kidney Foundation, Japan (JKF 04-1).

References

- 1 Knapp HR: Dietary fatty acids in human thrombosis and hemostasis. *Am J Clin Nutr* 65(Suppl): 1687S-1698S, 1997.
- 2 Johnson RJ, Pritzl P, Iida H and Alpers CE: Platelet-complement interactions in mesangial proliferative nephritis in the rat. *Am J Pathol* 138: 313-321, 1991.
- 3 Hamazaki T, Tateno S and Shishido H: Eicosapentaenoic acid and IgA nephropathy. *Lancet* i: 1017-1018, 1984.
- 4 Donadio JV, Bergstrahl EJ, Offord KP, Spencer DC and Holley KE: A controlled trial of fish oil in IgA nephropathy. Mayo Nephrology Collaborative Group. *N Engl J Med* 331: 1194-1199, 1994.
- 5 Folch J, Lees M and Sloane Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497-509, 1957.
- 6 Nakamura N, Hamazaki T, Yamazaki K, Taki H, Kobayashi M, Yazawa K and Ibuki F: Intravenous infusion of tridocosahexaenoyl-glycerol emulsion into rabbits. Effects on leukotriene B_{4/5} production and fatty acid composition of plasma and leukocytes. *J Clin Invest* 92: 1253-1261, 1993.
- 7 James MJ, Gibson RA and Cleland LG: Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr* 71(Suppl): S343-348, 2000.
- 8 Cho HP, Nakamura MT and Clarke SD: Cloning, expression, and fatty acid regulation of the human D-5 desaturase. *J Biol Chem* 274: 37335-37339, 1999.
- 9 Fujita T, Nakamura N, Kumasaka R, Shimada M, Murakami R, Osawa H, Yamabe H and Okumura K: Comparison of lipid and fatty acid metabolism between minimal change nephrotic syndrome and membranous nephropathy. *In Vivo* 20: 891-894, 2006.

Received November 24, 2006

Accepted December 14, 2006