Thiobarbituric acid reactive substances are increased in the subcutaneous fat tissue of patients with end-stage renal disease

M. Gotoh¹, S. Nagase¹, K. Aoyagi¹, A. Hirayama, K. Takemura¹, A. Ueda¹, C. Tomida¹, H. Kikuchi² and A. Koyama¹

¹Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba; ²Dialysis Unit, Department of Internal Medicine, Tsukuba Gakuen Hospital, Ibaraki, Japan

Abstract

Background. Patients with end-stage renal disease are thought to be in a highly peroxidative state, based on studies showing decreased serum antioxidant activity and increased peroxidative products. In this study we confirm these findings by examining lipid peroxidation in subcutaneous fat tissue in uraemia.

Subjects and methods. Twenty-seven subcutaneous fat samples were taken from patients with end-stage renal disease when they underwent intervention for arteriovenous fistula for haemodialysis or for catheter insertion for continuous ambulatory peritoneal dialysis. The control samples were taken from 11 patients with normal renal function and without any history of renal disease who had surgical interventions. Lipid peroxides were measured as thiobarbituric acid reactive substance.

Results. The concentration of thiobarbituric acid reactive substances in subcutaneous fat tissue in the patients with end-stage renal disease is 1.223 ± 0.636 nmol/mg fat tissue (mean ± SD) whereas the level in the control group is 0.097 ± 0.054 nmol/mg fat tissue. A comparison of the two groups by Student’s t test revealed a highly significant difference (P < 0.001).

Conclusions. This study supports the finding of a severe peroxidative state in patients with end-stage renal disease.

Key words: end-stage renal disease; fat tissue; haemodialysis; lipid peroxidation; thiobarbituric acid reactive substance

Introduction

Lipid peroxidation has been shown to be involved in the pathogenesis of a variety of diseases such as inflammation, ischaemia, atherosclerosis, cancer, heart disease, and stroke [1–3]. In a previous study we found that methylguanidine, a potent uraemic toxin which is present in increased amounts in the plasma of uraemic patients, is a peroxidative product of creatinine and is synthesized in the hepatic microsomal fraction [4–8]. In addition, we also reported that the generation rate of thiobarbituric acid reactive substances (TBARS) in microsomes obtained from rats with experimental renal failure was significantly higher than that of controls [7], and is associated with a decreased serum antioxidant activity in haemodialysis (HD) patients as demonstrated by a less inhibitory effect of uraemic serum on microsomal generation of TBARS, a marker of lipid peroxidation [8]. Accordingly, this condition is thought to be prevalent among patients with end-stage renal disease (ESRD) [9–13]. However, our previous study revealed that the concentration of plasma TBARS did not correlate with methylguanidine [14]. It was previously reported that plasma TBARS levels in patients with ESRD on maintenance HD was higher than those in healthy controls [9,12]. Another study noted that the TBARS level in the red blood cell membranes in HD patients is higher than that of healthy controls [10]. However, no significant difference in the level of plasma TBARS between uraemics and controls is also reported [13], leaving the issue of plasma TBARS and uraemia in dispute. The aim of the present study is to investigate the peroxidative state in the patients with ESRD from the view point of the lipid peroxide content in subcutaneous fat tissue, which is rich in polyunsaturated fatty acids, a normal substrate for TBARS.

Subjects and methods

Subjects

Twenty-seven ESRD patients (15 males, 12 females) with a serum creatinine concentration of more than 3.0 mg/dl (9.1 ± 3.2 mg/dl, mean ± SD), aged 23–76 years (57.4 ± 13.8 years, mean ± SD) and 11 individuals with normal renal function were examined. The aetiologies of ESRD were diabetic nephropathy in eight patients, chronic glomerulo-
nephritis in 11, nephrosclerosis in two, polycystic kidney in one, gouty kidney in one, and unknown in four.

The twenty-seven ESRD patients were divided into three groups based on the exposure to dialysis.

A: Patients in the predialysis stage. Thirty patients without severe clinical signs or symptoms of uraemia were managed on conservative treatment. At the time of study, nine patients had undergone surgery for creation of an arteriovenous fistula and all nine started dialysis within 3 months after surgery. The remaining four subjects subsequently underwent insertion of an intraperitoneal catheter for continuous ambulatory peritoneal dialysis (CAPD).

B: Patients at the initiation phase of regular dialysis treatment. Seven patients with severe clinical signs or symptoms of uraemia were at the start of regular dialysis treatment (RDT). All of them had commenced emergency HD by double-lumen catheters within 1 month before they underwent surgery for arteriovenous fistulae. The seven patients at the initiation phase of RDT were dialysed three times weekly with cuprophane membranes (0.8 m²), each session lasting 3 h. The dialysate used was kindly AF-2 (bicarbonate-based, Fuso Pharmaceutical Co., Osaka, Japan).

C: Patients undergoing regular dialysis treatment. The other seven patients were undergoing RDT with a mean duration of 57.3 months (22–120 months). They were clinically stable except for shunt troubles, at which time they underwent surgery for new arteriovenous fistulae (n = 4) or catheter insertions for CAPD (n = 2). The patients undergoing RDT were dialysed three times weekly, each session lasting 3 or 4 h. The dialysers were equipped with polysulphone (n = 3), cuprophane (n = 2), and polymethylmethacrylate (n = 2) membranes (1.5–2.1 m²). The dialysate used was kindly AF-2.

D: Controls. As controls, 11 individuals (9 males, 2 females) with serum creatinine concentration of less than 1.0 mg/dl (0.8 ± 0.1 mg/dl, mean ± SD) aged 28–78 years (53.0 ± 20.7 years, mean ± SD) who had surgical operation (lung cancer in 7, wryneck in 1, myasthenia gravis in 1, arthritis deformans of the left elbow in 1, giant-cell tumour of the left radius bone in 1) were examined. Both of the groups were informed of the aims of the study, and we obtained specimens after getting written consent from the patients.

Specimens and analytical procedures

Fat specimens were taken from the ESRD patients when they underwent interventions for arteriovenous fistula (n = 21) or for insertion of a catheter for CAPD (n = 6). Fat specimens from the controls were taken when they had other miscellaneous surgical interventions. Samples were kept frozen at −70 °C until used for the measurement of TBARS [15].

Blood samples were drawn from the arterial side of the arteriovenous fistula of patients undergoing RDT before HD and from the anteecubital veins of the other ESRD patients and controls. Serum total protein, albumin, urea nitrogen, creatinine, uric acid, GOT, GPT, LDH, alkaline phosphatase, total and HDL cholesterol, triglyceride, glucose, total calcium, inorganic phosphorus, Na, and Cl were determined by an autoanalyzer (Hitachi 736–15, Japan). Serum iron was measured colorimetrically and serum ferritin by an enzyme immunoassay. Red and white blood cells, haemoglobin, haematocrit, and platelets were determined by an autoanalyzer (Coulter STKS, USA). TBARS and methylguanidine concentration in the serum of ESRD patients were measured according to methods previously reported [4,15].

Statistical analysis

Results are presented as the mean ± standard deviation (SD). Statistical analysis was performed by an analysis of variance, and Student’s t test for unpaired variables. A P value less than 0.05 was considered significant.

Results

Comparison of TBARS between the ESRD group and the control group

As shown in Figure 1, the concentration of TBARS in subcutaneous fat tissue in the ESRD group (n = 27) was 1.223 ± 0.636 nmol/mg, whereas the level in the control group (n = 11) was 0.097 ± 0.054 nmol/mg. There was a statistically significant difference between the two groups (P < 0.001). The concentrations of serum urea nitrogen and serum creatinine were 87.4 ± 32.4 and 9.1 ± 3.2 mg/dl in the ESRD group respectively, and 15.9 ± 3.4 and 0.8 ± 0.1 mg/dl in the control group, as shown in Table 1. There were statistically significant differences between the two groups (P < 0.001) in serum urea nitrogen and serum creatinine levels. There was no significant difference in age.

Comparison of TBARS between diabetic and non-diabetic groups

There was no significant difference between the diabetic group (n = 8) and non-diabetic group (n = 19) in the TBARS levels of subcutaneous fat tissue as shown in Figure 2. There was no significant difference in age,
Increased TBARS in the fat tissue of patients with ESRD

Table 1. Characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=11)</th>
<th>Patients with ESRD (n=27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.0±20.7</td>
<td>57.2±13.8</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>9/2</td>
<td>15/12</td>
<td></td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td>15.9±3.4</td>
<td>87.4±32.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.8±0.1</td>
<td>9.1±3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>169.0±28.2</td>
<td>179.7±52.0</td>
<td>NS</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>149.3±90.4</td>
<td>152.1±57.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

There are statistically significant differences between two groups in serum urea nitrogen and creatinine levels (P<0.001) and no significant differences in age, serum total cholesterol, and triglyceride levels. Values are expressed as mean±SD. NS, not significant.

Table 2. Characteristics of the patients with end-stage renal disease distinguishing diabetics and non-diabetics

<table>
<thead>
<tr>
<th></th>
<th>DM (n=8)</th>
<th>non-DM (n=19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.3±6.2</td>
<td>55.5±15.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>4/4</td>
<td>11/8</td>
<td></td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td>78.8±36.4</td>
<td>91.6±30.8</td>
<td>NS</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>6.9±2.3</td>
<td>9.9±3.2</td>
<td>NS</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>221.7±73.7</td>
<td>164.0±31.6</td>
<td>NS</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>199.3±60.7</td>
<td>134.7±46.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

There are no significant differences in age, serum urea nitrogen, creatinine, total cholesterol, and triglyceride levels between the two groups. Values are expressed as mean±SD. NS, not significant; DM, diabetic; Non-DM, non-diabetic.

Influence of the exposure to dialysis

The ESRD patients were divided into three groups based on the exposure to dialysis, i.e. patients in the predialysis stage on conservative treatment (n=13), patients at the initiation phase of RDT (n=7), and patients undergoing RDT (n=7). There was a tendency for the levels of TBARS in the patients undergoing RDT to be lower than those in the patients at the initiation phase of RDT and those in the predialysis stage, though there were no significant differences among the three groups (Figure 3). As shown in Table 3, there were also no significant differences in age, serum urea nitrogen, and serum creatinine among the three groups.
The patients with end-stage renal disease (ESRD) were divided into three groups based on the exposure to dialysis, i.e., patients in the predialysis stage on conservative treatment, patients at the initiation phase of regular dialysis treatment (RDT), and patients on RDT. There are no significant differences in age, serum urea nitrogen, creatinine, total cholesterol and triglyceride levels among the three groups.

### Correlations among serum TBARS, methylguanidine, and TBARS in fat tissue in the ESRD group

TBARS levels in fat tissue in the ESRD group did not correlate with serum levels of TBARS ($r = -0.023$) or methylguanidine ($r = -0.141$) (not shown).

### Discussion

Recent studies have revealed that uraemic patients are in a highly peroxidative state [1,2] in addition to other pathological conditions. Several markers which predict this peroxidative tendency are proposed such as conjugated diene [16], antioxidative enzymes [17], methylguanidine as a peroxidative product of creatinine [4,6], serum antioxidant activity [8,11], and lipid peroxides [9–14,16–19]. In all these markers, lipid peroxides are thought to reflect reliably the peroxidative tendency and are employed in several studies. In this study we focused on subcutaneous fat tissue, since it is questionable whether the level of TBARS in plasma reflects the redox state in vivo.

Our findings show that the TBARS level in subcutaneous fat tissue of ESRD patients is also high as compared to that in serum and red blood cells. One possible mechanism for this observation is that the lipid peroxides are produced in subcutaneous fat tissue itself and products of TBARS are increased because serum antioxidant activity in patients with ESRD is decreased [8,11]. Another possibility is that the lipid peroxides produced in other organs may accumulate in fat tissue transported via circulating plasma. Since we have already reported that lipid peroxidation by rat liver microsomes is increased in renal failure [7], the increase in TBARS levels in subcutaneous fat tissue of patients with ESRD directly implies that antioxidant activity is decreased in patients with ESRD.

No significant differences in the TBARS levels of subcutaneous fat tissue were found between the diabetic and non-diabetic ESRD patients, although the TBARS levels in both groups were significantly higher than those in controls. In fact, TBARS levels of the diabetic group tend to be lower than those of the non-diabetic group. There are no reports comparing TBARS levels in serum or other tissues between diabetic and non-diabetic ESRD patients. Generally speaking, diabetic patients seem to be in a hyperoxidative state and this tendency could bring about atherosclerosis and vascular damage. Our findings do not support this speculation. One possible mechanism for a decreased vulnerability to oxygen damage in the diabetic group is the protection afforded by high concentrations of glucose in the circulating blood.

In this study no significant differences were found among the three groups divided according to the duration of HD; however, TBARS levels of patients undergoing RDT tend to be lower than those of patients at the initiation phase of RDT and those of patients in the predialysis stage on conservative treatment. This is in agreement with our hypothesis that haemodialysis improves the antioxidant activity of patients undergoing RDT [9]. This study did not address the role played by various dialyser membranes, whose differences in permeability and biocompatibility might be important. This could well be the subject of an expanded series of observations.

As we reported previously, methylguanidine did not correlate with TBARS in the serum of ESRD patients [14]. Serum methylguanidine levels in this study also do not correlate with TBARS levels in fat tissue. One possible explanation is that the species of reactive oxygen involved in methylguanidine synthesis and lipid peroxidation is different. The species concerned with methylguanidine synthesis is the hydroxyl radical [4], while it is thought to be singlet oxygen [20] in lipid peroxidation. Furthermore, serum TBARS levels did not correlate with those in fat tissue. We reported that serum TBARS levels do not vary much, and even in ESRD patients the values are almost within the normal range [14]. One of the reasons we measured TBARS in fat tissue in this study is the variability noted in the literature comparing serum TBARS in controls and ESRD subjects [9,12,13].

This study shows that TBARS levels in fat tissue are significantly higher in ESRD patients than in healthy controls and that the values do not correlate with serum levels. Serum TBARS levels could be kept low by some protective mechanisms such as hepatic microsomes which, as we have indicated, are capable of both TBARS generation and degradation [7].

### Table 3. Characteristics of the patients divided into three groups based on the exposure to dialysis

<table>
<thead>
<tr>
<th></th>
<th>ESRD patients in the predialysis stage ($n=13$)</th>
<th>ESRD patients at the initiation phase of RDT ($n=7$)</th>
<th>ESRD patients on RDT ($n=13$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.4 ± 13.7</td>
<td>66.1 ± 15.2</td>
<td>54.6 ± 10.2</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>8/5</td>
<td>3/4</td>
<td>4/3</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td>98.2 ± 27.6</td>
<td>73.1 ± 42.7</td>
<td>80.5 ± 24.4</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>9.0 ± 2.7</td>
<td>7.5 ± 3.5</td>
<td>11.1 ± 3.4</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>178.6 ± 48.5</td>
<td>170.4 ± 17.0</td>
<td>192.8 ± 71.4</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>149.6 ± 40.1</td>
<td>166.3 ± 83.0</td>
<td>141.0 ± 61.1</td>
</tr>
</tbody>
</table>
Increased TBARS in the fat tissue of patients with ESRD

TBARS levels in fat tissues may be a more sensitive index of the peroxidative tendency because of the abundance of substrate for TBARS generation in situ and/or the accumulation of TBARS generated in other organs transported via circulating blood.

The peroxidative state of ESRD patients demonstrated in this study confirms our previous report that serum antioxidant activity in HD patients is decreased [9]. Decreased serum antioxidant activity is possibly one of causes of the increased lipid peroxidation shown in subcutaneous fat tissue in this study.

Acknowledgements. We are deeply indebted to Professor B. D. Cohen for his valuable criticism in preparing the manuscript. This study was supported in part by a research grant from Public Health Bureau, Ministry of Health and Welfare, Japan and the Research Project of the University of Tsukuba.

References


Received for publication: 8.7.96
Accepted in revised form: 3.12.96