Increased Urinary Lipoperoxide Levels in Renal Transplant Patients

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ABSTRACT

Using high performance liquid chromatographic methods, both plasma and urine lipoperoxide concentrations were measured, as malondialdehyde (MDA), in 30 stable renal transplant patients receiving daily cyclosporine and/or azathioprine therapy. Their MDA concentrations were compared with previously reported reliable reference values using the same liquid chromatographic methods. Although their plasma concentrations were within the reference range, their mean urine MDA values averaged 3.7 to 5.0 times the normal reference values (p < 0.001). The primary cause of the increased urine MDA concentrations following renal transplantation in these patients is unknown; it could be due to (a) renal lipid peroxidation directly related to the cyclosporine/azathioprine therapy, (b) drug-induced or other nephrotoxicity by an alternative mechanism with secondary lipid peroxidation, (c) increased lipid peroxidation owing to an immunologic response to the kidney graft, or (d) a combination of these possibilities.

Introduction

Oxidative damage to polyunsaturated lipids in tissue membranes (lipid peroxidation), a free radical process, is a widely accepted mechanism for cellular injury. It was initially shown to represent the primary mechanism of cellular injury following ionizing radiation. It has been related to various toxic substances including certain solvents (CCl₄), drugs (paracetamol), pesticides (paraquat), metals (Fe, Cu, Ni, etc.), and, more recently, to drugs of abuse (cocaine, opiates, etc.).

The possibility of increased lipid peroxidation was investigated in stable renal transplant patients receiving daily cyclosporine and/or azathioprine therapy. Their plasma and urine lipoperoxide...
concentrations were measured using high performance liquid chromatographic (HPLC) methods and compared with recently published reference values quantitated by the same techniques.

**Methods and Materials**

**Patients**

Thirty patients (23 males, 7 females), two to 44 months post-renal transplantation without acute intercurrent illness, were studied. They had normal or near normal function of the renal grafts, as indicated by their serum urea nitrogen and creatinine concentrations [mean serum urea nitrogen 26.4 mg per dl (9.4 mmol per L), range 15 to 60 mg per dl (5.4 to 21.4 mmol per L); creatinine mean 1.7 mg per dl (150 μmol per L), range 0.8 to 2.6 mg per dl (71 to 230 μmol per L)]. They were all on daily prednisone (7.5 to 20 mg). The patients were divided into two groups: (a) those on cyclosporine with azathioprine (17 patients) or without azathioprine (4 patients), and (b) those receiving only azathioprine (9 patients). Cyclosporine dosage varied form 130 to 600 mg per d while azathioprine varied form 50 to 100 mg per d. Serum cyclosporine concentrations were maintained below the presumed toxic range (trough level less than 100 μg per L). A variety of other medications were also prescribed. All patients had normal serum liver function tests (bilirubin, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase).

**Lipoperoxides**

Plasma lipoperoxides were performed by the HPLC method of Wong et al; urinary malondialdehyde concentrations were measured using a modification of the plasma HPLC method, as recently described. Reliable plasma and urine reference values of MDA determined on large groups of both men and women, using these same analytical methods, have been recently reported.

**Statistics**

Data computations included the means (m), range, standard deviations (SD), and students's unpaired t-test.

**Results**

**Plasma Lipoperoxides**

The mean (and SD) plasma values of MDA from the 21 transplant patients taking cyclosporine, with and without azathioprine, were 0.62 μmol per L (0.23), while those on azathioprine alone were 0.58 (0.16) μmol per L (table I). These concentrations were essentially identical with the reference values previously reported. All measurements were within the reference range except for one patient with hyperlipidemia (serum triglycerides 514 mg per dL, [5.8 mmol per L] as triolein; normal 0.3 to 1.8 mmol per L). The increased level of MDA in this case (1.23 μmol per L) was not unexpected, since recent reports have shown that patients with high plasma lipid levels often have increased serum lipid peroxide concentrations.

**TABLE I**

<table>
<thead>
<tr>
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<th>Plasma MDA (μmol/L)</th>
<th>Urine MDA (nmol/mg Cr)</th>
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<tbody>
<tr>
<td>Cyclosporine patients</td>
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<tr>
<td>(n = 21)</td>
<td>0.62(0.23)*</td>
<td>3.32(1.57)†</td>
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<tr>
<td>Azathioprine patients</td>
<td></td>
<td></td>
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<tr>
<td>(n = 9)</td>
<td>0.58(0.16)*</td>
<td>4.15(3.03)†</td>
</tr>
<tr>
<td>Reference values</td>
<td>0.58(0.23)†</td>
<td>0.89(0.35)‡</td>
</tr>
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</table>

*p<0.05 vs. normal.
‡p<0.001 vs. normal.
Urine Lipoperoxides

The urine lipoperoxide values from the transplant patients were significantly elevated above the reference levels (p < 0.001) (table I); 17/21 of the patients receiving cyclosporine had MDA concentrations above the upper normal value of 1.59 nmol per mg Cr (group range 0.85 to 6.43 nmol per mg Cr), while three of the remaining four exceeded the normal mean. The four patients receiving cyclosporine without simultaneous azathioprine had concentrations of MDA in urine similar to those who were taking both drugs (i.e., 2.32, 3.99, 4.82, and 6.43 nmol MDA per mg Cr). The mean (SD) concentration of MDA in urine for these 21 patients was 3.32 (1.57) nmol per mg Cr, compared with the reference mean (SD) of 0.89 (0.35) nmol per mg Cr. Of the nine patients receiving only azathioprine, eight had elevated levels of MDA. Their mean (SD) and range were 4.15 (3.03) and 0.55 to 10.95 nmol per mg Cr, respectively.

Discussion

Oxidative damage to polyunsaturated lipids has been extensively studied in laboratory animals over the past 20 years. More recently, increased lipid peroxidation has been noted in a wide variety of clinical and toxicological conditions. However, the demonstration that the presence of disease or toxin is accompanied by increased lipid peroxidation is not unequivocal evidence that lipid peroxidation causes the damaging cellular effects. In this regard, it has been recently suggested that current evidence for lipid peroxidation producing cellular damage is strongest in cancer and rheumatic joint disease. On the other hand, it may be that the observed increased lipid peroxidation is merely the consequence of the disease/toxin. The disease/toxin may cause cellular injury by another mechanism; the damaged cells then become more susceptible to lipid peroxidation.

In the current report, highly sensitive and specific HPLC methods were used to measure both plasma and urine lipoperoxides, as MDA, in patients who had undergone renal transplantation and were receiving cyclosporine and/or azathioprine daily. Based on the results of this study, the following conclusions are offered.

1. Plasma levels of lipoperoxides were normal in the renal transplant patients receiving oral cyclosporine and/or azathioprine.

2. Although cyclosporine is potentially both hepatotoxic and nephrotoxic, there was no apparent hepatotoxicity in these patients since both plasma levels of MDA and liver function tests were normal.

3. There was a significant increase in urinary lipoperoxides in the majority of transplant patients (23/30), regardless of whether they were receiving cyclosporine, cyclosporine plus azathioprine, or azathioprine alone. In those with normal values (7/30 patients), all but two exceeded the normal mean, and most were in the upper quartile.

4. In the absence of elevated plasma values of MDA, the cause of the increased lipid peroxidation is presumably related to a renal etiology. This could be due to cyclosporine/azathioprine nephrotoxicity, either primarily by lipid peroxidation and/or as a secondary phenomenon, i.e., initial nephrotoxicity by an alternative mechanism; the injured cell being secondarily susceptible to increased lipid peroxidation. It could also be due to low-grade subclinical immunologic response to the graft. This latter suggestion is a distinct possibility since it has been postulated that
l lipid peroxidation may lead to tissue damage in autoimmune disorders, especially rheumatoid arthritis.4

5. This preliminary report raises several questions which should lead to fruitful investigation. Further studies are needed to clarify the relationship between lipid peroxidation and cyclosporine/azathioprine toxicity and the immune effects of tissue rejection.

Addendum

Since submission of this study for publication, P. D. Walker and S. V. Shah have briefly reported data that suggest a possible role for reactive oxygen metabolites in cyclosporine-induced renal tubulo-interstitial injury (cyclosporine enhances lipid peroxidation in renal cortical mitochondria. United States and Canadian Academy of Pathology, Annual Meeting, San Francisco, CA, March 5–10, 1989. Abstract #610).

References


