Mycophenolate Mofetil Treatment Improves Hypertension in Patients with Psoriasis and Rheumatoid Arthritis

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Evidence that was obtained in several experimental models and in strains of hypertensive rats indicates that infiltration of inflammatory cells and oxidative stress in the kidney play a role in the induction and maintenance of hypertension. Similar evidence is lacking in human hypertension, at least in part, because immunosuppressive treatment is unjustified in patients with hypertension. For addressing this issue, patients who were prescribed by their private physicians mycophenolate mofetil (MMF) for the treatment of psoriasis or rheumatoid arthritis and had, in addition, grade I essential hypertension and normal renal function were studied. Eight patients were studied before MMF was started, during MMF treatment, and 1 mo after MMF treatment had been discontinued. Other treatments and diet were unchanged in the three phases of the study. MMF therapy was associated with a significant reduction in systolic, diastolic, and mean BP. Urinary excretion of TNF-α was reduced progressively by MMF treatment and increased after MMF was discontinued. Reduction of urinary malondialdehyde, TNF-α, and RANTES excretion during MMF administration did not reach statistical significance but had a direct positive correlation with the BP levels. These data are consistent with the hypothesis that renal immune cell infiltration and oxidative stress play a role in human hypertension.

Hypertension is the estimated cause of 7.1 million premature deaths and 64 million disability-adjusted life years lost worldwide (1). Multiple factors play a role in the development and maintenance of essential hypertension. Among them, the role of the kidney in driving a tendency to salt retention represents one of the most studied and debated conditions in the hypertensive patient (reviewed in reference [2]).

We previously postulated that renal tubulointerstitial inflammation, in association with oxidative stress and intrarenal angiotensin activity, represents a final common pathophysiologic pathway that induces and sustains salt retention (3–6). This postulate is based on the demonstration that accumulation of immunocompetent cells, increased renal oxidative stress, and angiotensin II activity are a feature of all experimental models thus studied far and that immunosuppressive anti-inflammatory therapies ameliorate or prevent hypertension in those models (7–12). Particularly compelling are the studies in spontaneously hypertensive rats that become normotensive with mycophenolate mofetil (MMF) treatment (13) and, furthermore, fail to develop hypertension if early and sustained inhibition of proinflammatory transcription NF-κB is induced (14).

Although the evidence in experimental models is convincing, no studies in humans have confirmed that inflammatory reactivity in the kidney participates in the pathogenesis of essential hypertension. There are several reasons for the lack of human data: First is that immunosuppressive drugs that are used in animal studies, such as MMF, have potentially severe adverse effects despite the relative safety that has been reported in long-term studies (15,16). Therefore, its administration is unjustified in uncomplicated hypertension, a condition in which other medications provide safe and effective treatment.

Second are the difficulties of designing studies of BP modifications in patients who receive immunosuppressive treatment for diseases that are associated with hypertension. Transplant patients who receive tacrolimus-based therapy have less hypertension than cyclosporine-treated patients (17,18), and combinations of immunosuppressive agents that include MMF and rapamycin are associated with less incidence of hypertension (reviewed in reference [19]). However, although is clear that drugs such as MMF and rapamycin do not have hypertensive effects, it is not possible to conclude that these drugs actually are capable of ameliorating hypertension. Furthermore, findings in renal transplant recipients may not be extrapolated to patients with essential hypertension. When MMF is given as a treatment for immune-related diseases that compromise renal function, lupus nephritis for example, it is difficult or impossible to separate any beneficial effects on BP from improvement in the renal function that results from the treatment.

The present studies were designed to gain insight into the possible antihypertensive effects of MMF in patients with essential hypertension. Because of the difficulties mentioned previously, we selected a group of patients who did not have...
significant kidney disease, had grade I essential hypertension, and were prescribed MMF by their own doctors for the treatment of two conditions in which this therapy sometimes is indicated: Rheumatoid arthritis and psoriasis. These patients were studied before, during, and after MMF therapy. We found that 3 mo of MMF treatment resulted in a reduction in arterial pressure and urinary TNF-α and RANTES urinary excretion without detectable changes in salt or protein intake or renal function. These findings are compatible with the postulate that intrarenal inflammation participates in the pathogenesis of essential hypertension.

Materials and Methods

Patients

The study was done in patients who had grade I essential hypertension (140 to 159/90 to 99) and normal renal function and were prescribed MMF by their personal doctors for the treatment of psoriasis or rheumatoid arthritis. Exclusion criteria were history of renal disease, diabetes, or calcui; serum creatinine >1.2 mg/dl; symptoms of urinary tract infection or urinary sediment presenting leukocyturia or bacteriuria; and proteinuria >200 mg/d. The treatment of the patient remained under the care of the referring physician, and the patients consented to specific follow-up intervals in the Renal Unit in which history, physical examination (including BP, see Study Design), and blood and urine samples were collected for studies. Diet and other medications were to be unchanged during the study.

Eight patients (five women) who ranged in age from 50 to 65 yr gave their informed consent. Three patients had psoriasis, and five patients had rheumatoid arthritis. Before the study, four patients were taking methotrexate that was discontinued 2 wk before the baseline studies.

Table 1. Characteristics of the patientsa

<table>
<thead>
<tr>
<th>Patient (Age, Gender)</th>
<th>Clinical Diagnosis</th>
<th>Medicationsb</th>
<th>SBP/DBP (mmHg)</th>
<th>Urine</th>
<th>Serum Creatinine (mg/dl)</th>
<th>MMF Dosage (g/d)</th>
<th>Treatment Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (53, M) Psoriasis</td>
<td>None</td>
<td>146/90</td>
<td>Proteinuria 125 mg/d, sediment normal</td>
<td>0.6</td>
<td>1.5</td>
<td>Mild improvement of psoriasis</td>
<td></td>
</tr>
<tr>
<td>2 (52, M) Psoriasis</td>
<td>None</td>
<td>148/89</td>
<td>Proteinuria 130 mg/d, sediment normal</td>
<td>0.9</td>
<td>1.5</td>
<td>Psoriasis unchanged</td>
<td></td>
</tr>
<tr>
<td>3 (65, F) Rheumatoid arthritis</td>
<td>Prednisone 5 mg OD, HCT 50 mg/d</td>
<td>159/90</td>
<td>Proteinuria 143 mg/d, sediment normal</td>
<td>0.7</td>
<td>1.5</td>
<td>Symptoms unchanged</td>
<td></td>
</tr>
<tr>
<td>4 (50, F) Rheumatoid arthritis</td>
<td>Prednisone 5 mg/d, ASA s.o.s.</td>
<td>146/89</td>
<td>Proteinuria 162 mg/d, sediment normal</td>
<td>0.7</td>
<td>1.5</td>
<td>Symptomatic improvement</td>
<td></td>
</tr>
<tr>
<td>5 (57, F) Rheumatoid arthritis</td>
<td>Chloroquine 2 tabs/d, Captopril 25 mg twice daily, ASA s.o.s.</td>
<td>159/97</td>
<td>Proteinuria 171 mg/d, sediment 99C 8-10/ hpf</td>
<td>0.6</td>
<td>2.0</td>
<td>Symptoms unchanged</td>
<td></td>
</tr>
<tr>
<td>6 (50, M) Rheumatoid arthritis</td>
<td>Prednisone 5 mg/d, HCT 50 mg/d, amiloride 5 mg/d, ASA s.o.s.</td>
<td>152/92</td>
<td>Proteinuria 131 mg/d, sediment normal</td>
<td>0.7</td>
<td>1.5</td>
<td>Mild symptomatic improvement</td>
<td></td>
</tr>
<tr>
<td>7 (54, F) Rheumatoid arthritis</td>
<td>Prednisone 5 mg/d, ASA s.o.s.</td>
<td>145/94</td>
<td>Proteinuria 140 mg/d, sediment normal</td>
<td>0.6</td>
<td>1.5</td>
<td>Symptoms unchanged</td>
<td></td>
</tr>
<tr>
<td>8 (58, F) Psoriasis</td>
<td>Amlodipine 10 mg/d, vitamins A, C, and D</td>
<td>157/90</td>
<td>Proteinuria negative, sediment normal</td>
<td>0.7</td>
<td>2.0</td>
<td>Mild improvement of psoriasis</td>
<td></td>
</tr>
</tbody>
</table>

aDBP, diastolic BP; HCT, hydrochlorothiazide; hpf, high-power field; RBC, red blood cells; SBP, systolic BP; OD, once daily; ASA, acetylsalicylic acid; S.O.S., as needed.
bMedications received during the study. Patients 3, 4, 5, and 6 received methotrexate that was discontinued 2 wk before they were included in the study. Vitamin E (400 mg/d) and vitamin C (500 mg/d), aspirin (2 to 8 tablets of 500 mg weekly, depending on joint symptoms).
need for incorporating additional medications for the control of psoriasis or rheumatoid arthritis (as determined by the referring physician). Modification of the antihypertensive treatment during the study was not a reason for withdrawal of the patient. All patients finished the study as planned.

**Laboratory Studies**

At each clinic visit, blood samples were obtained and 24-h urinary samples were analyzed. Urine collections were done at home, and the urine was kept refrigerated until arrival to the laboratory, where it was measured, aliquotted, and kept at −20°C until determinations were done. At each visit, the patients were asked about significant dietary changes, and 24-h urinary sodium and urea nitrogen excretion were used to evaluate sodium and protein intake, respectively (24). GFR was estimated from the serum creatinine values with the Modification of Diet in Renal Disease (MDRD) equation (25).

Routine hematology and blood chemistries were determined by the autoanalyzer method, and commercially available kits were used to determine plasma C-reactive protein (Bender Med Systems, Vienna, Austria; sensitivity 3 pg/ml), monocyte-chemoattractant protein-1 (MCP-1; R&D Systems, Minneapolis, MN; sensitivity 5.0 pg/ml), RANTES (Pierce Endogen, Rockford, IL; sensitivity 2 pg/ml), IL-6 (R&D Systems; sensitivity 0.11 pg/ml), and TNF-α (Research Diagnostics, Flanders, NJ; sensitivity 3 pg/ml). Urinary and plasma malondialdehyde (MDA) levels were analyzed by the method of Ohkawa et al. (26), as detailed in previous communications (27).

For avoidance of errors that are derived from unsupervised urinary collections, the MDA and cytokine are related to the urinary creatinine excretion. Trough blood levels of MMF were determined (HPLC) before the ingestion of the morning dose (C₀) on two occasions during the second month of administration of the drug.

**Statistical Analyses**

Repeated (paired) measures ANOVA were used to explore differences among the three periods of study. Significant differences were examined with Tukey-Kramer posttest. Two tailed $P < 0.05$ was considered significant.

Linear regression and Pearson correlation were used to establish the relationship between variables. Statistical analyses and graphs were done with commercially available programs (GraphPad Instat and GraphPad Prism, GraphPad Software, San Diego, CA).
Results

The characteristics of the patients are shown in Table 1. All patients had grade I hypertension despite the treatment (or lack of it) at the beginning of the study. Proteinuria was negative in one patient and <200 mg/d in the rest. The proteinuria remained essentially unchanged during and after MMF treatment (range 128 to 180 mg/d) and remained negative in patient 8 (Table 1). Microhematuria was found in one patient (patient 5; Table 1) in the initial evaluation and was an inconsistent finding subsequently, as it was present in only one of the follow-up visits.

Four patients (patients 1, 2, 4, and 7) were not receiving antihypertensive drug therapy, two patients (patients 3 and 6) were receiving a thiazide diuretic, and one patient each was taking captopril and amlopidine. Before the study, four patients were receiving a small dosage of prednisone (5 mg/d), and it was continued unchanged during all of the phases of the study. All of these medications remained unchanged during the study. All of the patients finished the study, and there were no recorded adverse effects of MMF treatment.

Table 2 shows the renal function, SBP and DBP, urinary Na excretion, and calculated protein intake during the study. Renal function and salt and protein intake were unchanged during the three phases of the study. SBP and DBP decreased progressively during MMF treatment, and in the third month of treatment, the reduction in BP reached statistical significance. As shown in Table 2, blood MMF levels (C<sub>M</sub>) were in the range that is considered therapeutic for immunosuppression in renal transplantation.

Figure 1 shows the individual changes in SBP, DBP, and mean BP during the study. There is a progressive reduction in the mean values of SBP, DBP, and mean BP. The reduction in BP is more pronounced after 3 mo, and at this time the effect is more consistent with respect to the SBP. To be noted, after MMF treatment was stopped, all patients had increments in SBP and all but one had increments in DBP.

Table 3 shows the plasma and urinary MDA and cytokine levels. C-reactive protein levels and urinary IL-6 and MCP-1 excretion did not have consistent changes. Plasma MDA and urinary excretion of MDA, RANTES, and TNF-α decreased during MMF treatment, but only the reduction in TNF-α excretion reached significant levels in relation to the immediate posttreatment values (Table 3).

There were positive linear correlations between the urinary TNF-α, RANTES, and MDA excretion and the mean BP and SBP levels (Figure 2). There also were positive correlations between the urinary excretion of MDA with the urinary excretions of TNF-α (Figure 3) and RANTES (r<sup>2</sup> = 0.0221, P < 0.01) and between the excretions of TNF-α and RANTES (Figure 4).

Discussion

Substantial experimental evidence indicates that tubulointerstitial infiltration of immunocompetent cells and intrarenal oxidative stress are relevant features in experimental models of hypertension and in genetic strains of hypertensive rats. These two conditions are interrelated, support one another, and combine to establish a tendency to sodium retention that favors the induction and maintenance of a hypertensive state (reviewed in references [3–6]). In the experimental animal, hypertension may be corrected or ameliorated with the administration of the immunosuppressive anti-inflammatory drug MMF as well as with other antioxidant and anti-inflammatory therapy (5,28–32); however, similar evidence is lacking in human hypertension because the effects of MMF on BP levels are difficult to separate from the effects that the drug may have on the renal conditions for which this drug is given. The use of immunosuppressive drugs is not justifiable in patients with uncomplicated essential hypertension; therefore, we choose to study patients in whom this medication was prescribed for the treatment of psoriasis or rheumatoid arthritis with the specific aims to define whether MMF treatment ameliorates hypertension and, if so, to uncover evidence that links this beneficial effect with improvement in renal inflammation. The urinary abnormalities that were present in some of our patients consisted of mild proteinuria (<200 mg/d in seven patients) and transient microhematuria in one patient; these abnormalities are common in patients with essential hypertension. Clearly, rheumatoid arthritis–associated renal disease cannot be excluded completely, but, if present, then it existed in association with a normal urinary sediment and normal GFR (Table 1).

### Table 3. Inflammatory cytokines<sup>a</sup>

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Pre</th>
<th>MMF</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Month 1</td>
<td>Month 2</td>
</tr>
<tr>
<td>Plasma CRP (mg/L)</td>
<td>3.05 ± 1.93</td>
<td>3.80 ± 2.43</td>
<td>3.51 ± 2.44</td>
</tr>
<tr>
<td>Plasma MDA (mg/L)</td>
<td>4.54 ± 6.71</td>
<td>2.07 ± 1.23</td>
<td>1.99 ± 0.97</td>
</tr>
<tr>
<td>Urine IL-6 (pg/mg Cr)</td>
<td>0.31 ± 0.21</td>
<td>0.22 ± 0.11</td>
<td>0.22 ± 0.10</td>
</tr>
<tr>
<td>Urine RANTES&lt;sup&gt;b&lt;/sup&gt; (pg/mg Cr)</td>
<td>21.40 ± 7.25</td>
<td>16.10 ± 9.21</td>
<td>15.70 ± 8.45</td>
</tr>
<tr>
<td>Urine MCP-1 (pg/mg Cr)</td>
<td>203.00 ± 250.00</td>
<td>128.00 ± 75.40</td>
<td>176.00 ± 113.00</td>
</tr>
<tr>
<td>Urine TNF-α&lt;sup&gt;b&lt;/sup&gt; (pg/mg Cr)</td>
<td>8.93 ± 1.83</td>
<td>8.40 ± 3.58</td>
<td>7.24 ± 2.79&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine MDA&lt;sup&gt;b&lt;/sup&gt; (nmol/mg Cr)</td>
<td>8.02 ± 12.90</td>
<td>3.55 ± 3.68</td>
<td>2.72 ± 1.42</td>
</tr>
</tbody>
</table>

<sup>a</sup>Cr, creatinine; CRP, C-reactive protein; MCP-1, monocyte-chemoattractant protein-1; MDA, malondialdehyde.

<sup>b</sup>Urine samples of only seven patients available for studies.

<sup>c</sup>P < 0.05 versus Post.
We chose to study urinary cytokines that are expressed both in the infiltrating cells and in tubular epithelial cells (33). Moreover, cross-talk between tubular epithelial cells and infiltrating cells modulates the local cytokine responses (34–36). Because others (7,8), as well as ourselves, have shown that NF-κB activation is an early feature (37) and plays a relevant role in hypertension in the SHR (14), we investigated proinflammatory cytokines that are stimulated by this transcription factor, such as MCP-1 and RANTES. TNF-α also is interesting because its production is increased in renal tubules by angiotensin II (38), and increased intrarenal angiotensin II is an important feature in many experimental models of hypertension (39), including interstitial nephritis (40).

It is recognized that the patients in this study were receiving insufficient treatment (or no treatment) for their grade I essential hypertension, especially because accepted treatment guidelines indicate that SBP in patients who are older than 50 yr ideally should be kept below 140 mmHg (1). This problem is not unique to the patients in this study; in the most recent report in the United States, only 59 and 34% of the patients with hypertension were treated and controlled, respectively (41). In Venezuela, the problem is much worse; available data indicate that only 39% of the patients are treated, and <10% have their hypertension controlled appropriately (42).

MMF treatment was prescribed to these patients by their referring physicians, who remained in charge of the patient treatment during the study. Initially, we planned to include ambulatory 24-h BP recordings, but several patients declined to use the monitoring devices because of the perception of increased discomfort caused by their psoriasis, in addition to the recognized mobility restrictions and disturbances in the patient’s and partners’ sleep associated with the procedure (43,44). Therefore, it was decided to base the study on determinations of BP in the outpatient clinic under carefully specified conditions using an automatic BP recording device.
The most frequent adverse effects of MMF therapy are diarrhea and vomiting and, less frequently, leukopenia and anemia. The patients in this study had no adverse symptoms or changes in the hematologic and biochemical parameters tested during the study, and all of the patients finished the study as planned. The patients were instructed to maintain their usual diet during the study, and, in fact, determinations of urinary sodium and urea nitrogen indicated that sodium intake and protein intake remained essentially unchanged (Table 2). As shown in Figures 1 and 2, two patients had an increment in BP the second month of MMF treatment. This was reported to their referring physician, who considered the possibility of increasing antihypertensive medication and saw them 1 wk later. At that time, both of the patients had 140/85 BP, and the physician decided to leave medication unchanged. We did not include these BP readings in the present study.

The 3-mo period that was chosen to evaluate potential beneficial effects of MMF turned out to be adequate for observing the effects of this drug on BP and urinary cytokine excretion. We had anticipated that the effects, if any, would be apparent after 1 mo of treatment, on the basis of the observations in SHR (13); although a tendency to a reduction in BP was evident after 1 mo, only after 3 mo did the reduction in BP and urinary TNF-α reached statistically significant levels. The reduction in the urinary excretion of RANTES (which did not reach statistical significance) also is somewhat unexpected because myco phenolic acid increases the production of RANTES in cultured human tubular epithelial cells (45).

The main findings of this work are, first, that MMF administration is associated with improvement in BP in patients with grade 1 essential hypertension (Figure 1) and, second, that modifications of BP are correlated with urinary excretion of TNF-α, RANTES, and thiobarbituric acid–reacting substances, reflecting oxidative stress (Figure 2). Because plasma MDA levels were not correlated with BP in our patients, it is tempting to suggest that intrarenal oxidative stress is a more critical long-term pro-hypertensive factor than systemic oxidative stress, but it must be recognized that MDA levels are a relatively insensitive measure of the oxidative stress, that the variability of pretreatment plasma MDA levels was very high (Table 3), and that the active psoriasis and rheumatoid arthritis are inflammatory conditions that may contribute to the systemic oxidant load of these patients.

There are limitations that need to be taken into account in relation to the interpretation of the results of the study. First is the unavoidably small number of patients, which makes it risky to extrapolate the findings to the large population of patients with essential hypertension. Second, it is conceivable that psychologic effects (expected benefit of therapy) could be a factor in the BP reduction during MMF treatment. Furthermore, improvement in the psoriasis or in the rheumatoid arthritis that was induced by MMF could have contributed indirectly (less pain or a reduction of anxiety) to the reduction in BP levels. In fact, four patients did experience improvement of their symptoms during MMF treatment (Table 1). However, the reported improvement was mild and the BP response was similar in those patients and in the patients who had unchanged symptoms; consequently, we consider unlikely that improvement of psoriasis or rheumatoid arthritis contributed significantly to the amelioration of hypertension. Another question that may be asked is whether the urinary cytokine excretion in normotensive patients with psoriasis or rheumatoid arthritis is modified by MMF treatment. Clearly, this question cannot be answered from the present study, but the correlation between BP levels and the urinary excretion of TNFα, RANTES, and MDA (Figure 2) suggests an association among these variables.

To our knowledge, there are no previous studies of the urinary cytokine excretion in patients with psoriasis or rheumatoid arthritis, but the serum levels of RANTES and TNF-α are increased in these patients (46–48). In contrast, uncomplicated essential hypertension is not associated with an increase in the serum levels of proinflammatory cytokines (49). Urinary excretion of RANTES and TNF-α were correlated directly with one another (Figure 4) and with urinary excretion of lipid peroxidation products (Figure 3).

It is widely recognized that MMF has actions on cells other than immune cells, but these results, in conjunction with the correlations with BP levels already mentioned and shown in Figure 2 and with experimental data that link renal inflammation and hypertension (8–14), suggest an association between the effects of MMF therapy on BP and its effects on intrarenal inflammation and oxidative stress. Although these observations do not constitute proof of a causal relationship, they nevertheless are consistent with the hypothesis that renal tubulointerstitial immune cell infiltration plays a role in the maintenance of elevated BP and represent, to our knowledge, the first such evidence, however indirect, obtained in patients with essential hypertension. Taken together with experimental data previously cited, the present findings underline the merit of additional investigations to define the role of renal inflammatory reactivity in essential hypertension.

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