Cyclosporin A Treatment in Patients with Primary Biliary Cirrhosis

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Summary: Six patients with primary biliary cirrhosis (PBC) were treated with a relatively low dose of cyclosporin A for more than 6 months to determine whether cyclosporin A is beneficial to PBC. Serum levels of cyclosporin A were maintained at 50-100 ng/ml during treatment in each patient. Serum levels of glutamate pyruvate transaminase, alkaline phosphatase and \( \gamma \)-glutamyl transpeptidase as well as serum levels of immunoglobulin M significantly decreased within 3 months following initiation of treatment, when compared with the pretreatment levels (p < 0.05, p < 0.01 p < 0.05 and p < 0.05, respectively). Histological changes in the liver before and after one year of treatment could be analysed in 2 of 6 patients, resulting in a marked reduction of infiltration of lymphocytes and OK-DR positive cells in the portal areas. However, cyclosporin A administration was discontinued within one year after initiation of treatment in 4 of 6 patients because of adverse effects such as hirsutism, renal dysfunction, impaired glucose tolerance, leukopenia and anemia. These results suggest that a beneficial effect of cyclosporin A in the treatment of PBC may be limited, because even a relatively low dose of cyclosporin A causes its well-known side effects in PBC patients.

Introduction

Primary biliary cirrhosis (PBC) is now diagnosed more frequently, partly owing to wider availability of laboratory screening such as serum levels of alkaline phosphatase (A1-P) and anti-mitochondrial antibody. In addition to the presence of the serum anti-mitochondrial antibody, DR-antigen expression on the bile duct epithelium also suggests PBC is an autoimmune liver disease. The Multicentric randomized therapeutic trials of immunosuppressive agents have been studied in PBC patients, but therapy of PBC is not satisfactory. Recently, the histopathological changes in the liver, which had a strong resemblance to nonsuppurative destructive cholangitis, were reported in patients with hepatic allograft rejection, promoting clinical therapeutic trials of cyclosporin A in PBC patients. Several groups have shown that cyclosporin A treatment of PBC induces biochemical and, in part, histological remission, although adverse effects were frequently observed.

In the present study, 6 patients with PBC were treated with a relatively lower dose of cyclosporin A than the doses used in the previous studies for more than 6 months to evaluate the clinical usefulness of cyclosporin A in the treatment of PBC.

Patients and Methods

Six patients with PBC were entered into this study. Informed consent was obtained from all 6 patients, and the study was approved by the ethical committee of the hospital. PBC was diagnosed on the basis of the histopathological findings of the liver in addition to the presence of high titers of the serum anti-mitochondrial antibody. All the patients were women, aged from 45 to 65 years (mean age: 53.5 years). Three patients were symptomatic; two had jaundice and one had generalized edema without jaundice. The remaining 3 patients were asymptomatic. The histopathological findings of the liver were classified according to Scheuer's classification. Three patients were in the early (I and II), and other 3 on the late stage (III and IV) of PBC before treatment.

Cyclosporin A (Sandimmun, Sandoz) was administered orally to all 6 patients for more than 6 months (mean duration: 10.5 months, range; from 6 to 16 months). The doses of cyclosporin A ranged from 3 to 5 mg/kg/day to maintain serum concentrations of cyclosporin A at 50-100 ng/ml during treatment.

Serum hepatic enzyme activities such as glutamate pyruvate transaminase (GPT), A1-P, and \( \gamma \)-glutamyl transpeptidase (\( \gamma \)-GTP) were measured by standard automated laboratory techniques, and serum levels of immunoglobulin M (IgM) were determined by a radial immunodiffusion method. The follow-up liver biopsies were performed in 2 of 6 patients one year after initiation of cyclosporin A treatment, using Silvermann's needles. Biopsy specimens were fixed in 10% formaldehyde solutions for hematoxylin-eosine (HE) staining, or fixed with 6% peridate-lysine-paraformaldehyde (PLP) and then frozen in liquid nitrogen for immunohistochemical staining. Serial frozen sections (approximately 6 um) were incubated with 0.3% H\(_2\)O\(_2\)-methanol solution for 10 min at room temperature to block endogenous peroxidase activities. The tissues were then reacted with mouse monoclonal antibodies to...
CD₃, CD₈, CD₁₄ or OK-DR (Coulter immunology, USA) diluted in 1% bovine serum albumin-0.1M phosphate buffered saline (antibodies to CD₃, CD₈ and OK-DR; 1:24, and antibodies to CD₁₄; 1:99) for 90 min at room temperature. Binding of these antibodies was identified with biotinylated antimouse IgG-peroxidase-conjugated streptavidin complexes (Biogenex Labo., USA). The color reaction was developed by diaminobenzidine.

Statistical analyses were performed using Student's t test. p-values less than 0.05 were considered statistically significant.

Results

Serial changes in serum hepatic enzyme activities and IgM levels

A decrease in serum Al-P activities was found within 3 months following initiation of treatment and the decreased levels were maintained during treatment in 4 of 6 patients. Serum Al-P activities did not decrease in the remaining 2 patients, but became markedly elevated after cyclosporin A was discontinued (Fig. 1a). Serum levels of γ-GTP were also reduced within 3 months after initiation of treatment in all 6 patients, and continued to be lower than the pretreatment levels during treatment in 4 of 6 patients. Marked elevation of serum γ-GTP levels was also found in 2 patients promptly after discontinuation of cyclosporin A (Fig. 1b).

Changes in serum hepatic enzyme activities and IgM levels after 3 months of treatment are summarized in Fig. 2. Serum enzyme activities of Al-P, γ-GTP and GPT significantly decreased during treatment, when compared with the pretreatment values (p < 0.05, p < 0.01 and p < 0.05, respectively). Serum levels of IgM during treatment were also significantly lower than the pretreatment levels (p < 0.05). Serum levels of both total bilirubin and albumin were unchanged during treatment (data not shown).

Histological changes in the liver

Histological changes in the liver before and after one year of treatment could be analysed in 2 patients. As shown in Fig. 3a and 3b, marked infiltration of mononuclear cells and pseudo-bile duct formations in the portal areas were found before treatment. Following one year of cyclosporin A treatment, infiltration of mononuclear cells was clearly reduced, although pseudo-bile duct formations still remained in the portal areas (Fig. 3c and 3d). By the analyses of the immunohistochemical staining of the liver, the number of OK-DR positive cells in the portal areas markedly decreased after one year of treatment, when compared with
Adverse effects

Although cyclosporin A treatment of PBC resulted in biochemical and histological remission, administration of cyclosporin A was discontinued within one year after initiation of treatment in 4 of 6 patients because of adverse effects. The adverse effects of cyclosporin A found during treatment are summarized in Table I. We found hirsutism in all 6 patients, renal dysfunction including proteinuria and the increased serum levels of creatinine in 4 patients, impaired glucose tolerance in 3 patients, hypertension and leukopenia in each of one patient, and anemia in 2 patients. These adverse effects disappeared within one month after discontinuation of cyclosporin A.

Table 1. Adverse effects of Cyclosporin A in the treatment of PBC

<table>
<thead>
<tr>
<th>Adverse effects</th>
<th>Hirsutism</th>
<th>Renal dysfunction</th>
<th>IGT</th>
<th>Hypertension</th>
<th>Leukopenia</th>
<th>Anemia</th>
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<td>4</td>
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</table>

IGT: Impaired glucose tolerance

Discussion

Many of immunosuppressive agents including corticosteroids, azathioprine, chlorambucil, and more recently, methotrexate have been extensively used to control the underlying pathogenesis of PBC. Corticosteroids induce biochemical and, in part, histological remission, but exacerbate osteoporosis associated with PBC. Administration of azathioprine was reported to have some beneficial effects on liver enzyme abnormalities, but had no effects on the histological progression of the liver. Furthermore, most immunosuppressive agents have been associated with substantial toxic side effects. In fact, chlorambucil therapy has been associated with bone marrow suppression, and methotrexate therapy has caused hepatic fibrosis and cirrhosis when used in the treatment of psoriasis and other conditions.

Other therapies evaluated for the treatment of PBC have included D-penicillamine, colchicine and ursodeoxycholic acid (UDCA). D-penicillamine therapy had been thought to be effective, but recent controlled studies have demonstrated that D-penicillamine has no value for the treatment of PBC. Colchicine therapy has had some beneficial effect, but the histological progression was not prevented. Similarly, UDCA therapy has been associated with clinical remission, however, the effect
of UDCA on histological progression and survival remains to be determined.

Since cyclosporin A has a strong immunosuppressive activity which represses progression of not only graft-versus-host disease but also a variety of autoimmune diseases,24,29 cyclosporin A has been recently used in the treatment of PBC.9,10,11,12.

In this study, 6 patients with PBC were treated with a relatively low dose of cyclosporin A, because a high dose of cyclosporin A is closely associated with severe side effects including nephrotoxicity and opportunistic infections.30,31,32,33. Serum hepatic enzyme activities as well as serum levels of IgM significantly decreased during treatment, when compared with the pretreatment values. Serum levels of A1-P or γ-GTP were elevated promptly after discontinuation of cyclosporin A in some patients. By the histopathological analyses of the liver in 2 patients, cyclosporin A treatment resulted in a marked reduction of inflammation of lymphocytes and OK-DR positive cells. These results suggest that cyclosporin A treatment induces both biochemical and histological remission in PBC patients. However, cyclosporin A administration had to be discontinued within one year after initiation of treatment in 4 of 6 patients because of various adverse effects including nephrotoxicity. Although the administration doses of cyclosporin A in this study were lower than those used in the previous studies,9,10,11,12 and serum levels of cyclosporin A were maintained at 50-100 ng/ml during treatment, adverse effects were found in all 6 patients. These results therefore suggest that the well-known side effects of even a relatively low dose of cyclosporin A render a limited clinical applicability in the treatment of PBC. Wiesner et al. also demonstrated that the treatment of PBC with a low dose of cyclosporin A resulted in improvement of liver enzyme abnormalities and prevention of the histological progression, but was associated with adverse effects as were observed in this study.10.

The high incidence of adverse effects with a relatively low dose of cyclosporin A remains to be elucidated, however, it is possible that PBC patients may predispose to the toxic effects of this agent. Some investigators have documented that the amount of biological active form of cyclosporin A is associated with alterations of lipid metabolism.34,35. Further studies are necessary to clarify the changes in cyclosporin A metabolism in PBC patients.

Acknowledgements

The author would like to thank Prof. Shigenobu Nagataki, the First Department of Internal medicine, Nagasaki University School of Medicine for his advice and revision and thank Dr. Keisuke Nakata and the doctors for the willing co-operation and technical assistance in this study.

References

19) Zachariae H, Kragballe K, Sgaard H. Methotrexate induced cirrhosis: histopathological analyses of the liver in 2 patients, cyclosporin A treatment resulted in a marked reduction of inflammation of lymphocytes and OK-DR positive cells. These results suggest that cyclosporin A treatment induces both biochemical and histological remission in PBC patients.


