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Effect of Mizoribine on IL-6 Release by Peripheral Blood Mononuclear Cells

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Key Words
Mizoribine · IgA nephropathy · Interleukin-6 · Mononuclear cells

Abstract
Background/Aim: A novel immunosuppressant, mizoribine (MZB), has recently been reported to be effective in the treatment of IgA nephropathy (IgAN), although its mechanism of action remains unknown. This study was conducted to investigate whether the efficacy of MZB on IgAN is exerted by suppression of interleukin-6 (IL-6) release. ***Methods:*** Peripheral blood mononuclear cells (PBMC) were collected from 4 children with IgAN (median age 13.0 years) and 4 control children (median age 5.2 years). PBMC were cultured with medium alone or medium with lipopolysaccharide (LPS), and then incubated with LPS and MZB. Culture supernatants were assayed for IL-6. ***Results:*** IL-6 release was increased by LPS in all subjects. Although the median value was higher in IgAN patients (median increase in IL-6 release 1,298.1%) than in controls (median 489.2%), statistical significance was not reached ($p > 0.05$). 10 mg/ml of MZB suppressed the release of IL-6 in both IgAN patients (median decrease in IL-6 release 39.3%) and controls (median 43.2%), with statistical significance ($p < 0.05$ and $p < 0.01$, respectively). ***Conclusion:*** This study suggests that MZB could suppress IL-6 release in vitro and thus may exert its efficacy on IgAN.

Introduction

Mizoribine (MZB) is a nucleoside of the imidazole class, which was isolated from the culture medium of the mold *Eupenicilliun brefeldianum* M-2166 in Japan [1]. As MZB was found to inhibit both humoral and cellular immunity by selectively inhibiting the proliferation of lymphocytes, it was developed as a new immunosuppressive agent [2]. Because of its safety and good tolerance in comparison with other immunosuppressants, MZB has been used in Japan for the prevention of renal transplantation rejection, for the treatment of lupus nephritis, rheumatoid arthritis and primary nephrotic syndrome [3].

Furthermore, in this journal we and others have recently reported that MZB can be used in the treatment of IgA nephropathy (IgAN) [4–6], although its mechanism of action has not been clarified.

It has been postulated that interleukin-6 (IL-6) is expressed by proliferating mesangial cells of diseased human glomeruli, and its levels are elevated in the urine of patients with mesangial proliferative glomerulonephritis including IgAN [7–9].

From these findings, we wonder whether the efficacy of MZB on IgAN is exerted by suppression of IL-6 release and wish to report the effect of MZB on IL-6 release by peripheral blood mononuclear cells (PBMC).

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Subjects and Methods

Four children with biopsy-proven IgAN (median age 13.0, range 7.1–16.2 years, 3 males, 1 female) were enrolled in this study. All children were found to have hematuria and/or proteinuria in an annual screening program for renal disease and were enrolled in this study within 3 years of onset. Although an anti-platelet agent was administered orally before entry to the present study, none were on angiotensin-converting enzyme inhibitors or non-steroidal anti-inflammatory drugs and none had a past history of steroid and immunosuppressive treatment including MZB. Four children admitted to our hospital for minor operations, such as undescended testes or inguinal hernia, served as controls (median age 5.2, range 2.8–12.8 years, 3 males, 1 female).

PBMC Isolation and Cultures

Blood (5 ml) was collected in sterile tubes containing 200 U preservative-free heparin from the 8 subjects (4 patients with IgAN and 4 healthy control children). The blood was diluted with an equal amount of RPMI 1640 containing glutamine (2 m*M*), HEPES (25 m*M*), gentamicin (50 pg/m1) and 5% heat-inactivated fetal calf serum (FCS). PBMC were prepared by Ficoll-Hypaque density centrifugation and washed three times. The viability of the cells was checked by trypan blue. The cells were resuspended at 1×10^6 cells/ml in RPMI 1640.

Cells were then cultured in flat-bottomed wells (1 ml/well) at 37°C in a humidified atmosphere of 5% CO_2 under diverse conditions, i.e. (1) medium alone; (2) medium with 500 ng/ml of lipopolysaccharide (LPS, Sigma, Poole, UK); (3) medium with 500 ng/ml of LPS and 0.1 mg/ml of MZB; (4) medium with 500 ng/ml of LPS and 1 mg/ml of MZB, and (5) medium with 500 ng/ml of LPS and 10 mg/ml of MZB. Culture supernatants were collected after 48 h and immediately stored at –70°C until lL-6 assay.

IL-6 assay

IL-6 levels were measured using a commercially available kit by chemiluminescent enzyme immunoassay (Human IL-6 CLEIA Fujirebio, Fujirebio, Tokyo, Japan). The detection limit of this assay was 0.2 pg/ml.

Results

Release of IL-6 by PBMC

The supernatants from PBMC cultured with medium alone or stimulated with 500 ng/ml LPS for 48 h were assayed for IL-6. IL-6 release from PBMC was increased by LPS in all subjects studied (median 599.6%, range 204.7–1,733.5%). Although the median value was higher in IgAN patients (1,298.1%) than in controls (median 489.2%), statistical significance was not reached ($p > 0.05$ by Mann-Whitney U test).

Effect of MZB on IL-6 Release by PBMC

We attempted to assess the ability of MZB to suppress the release of IL-6. The PBMC of patients with IgAN and controls were incubated with LPS and various concentrations of MZB. The result in each patient is expressed by the percent change in IL-6: (% change in IL-6) = (IL-6 released by PBMC incubated with LPS and MZB)/(IL-6 released by PBMC incubated with LPS alone) × 100%.

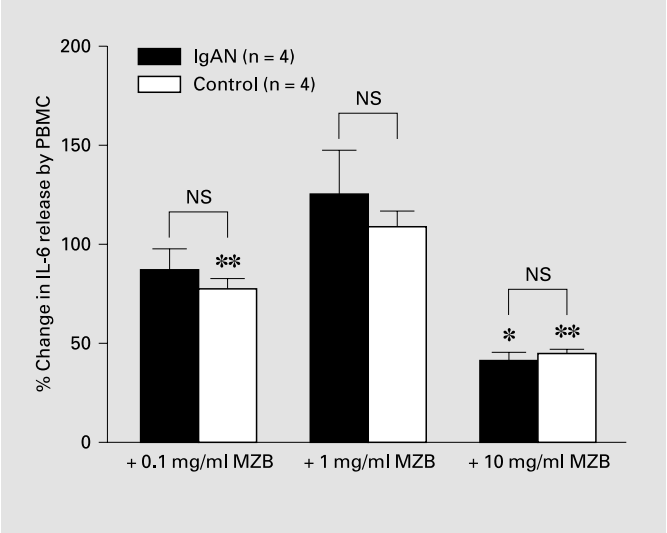

Fig. 1. Effect of MZB on IL-6 release by PBMC. Results are expressed as the mean percent change in IL-6: (IL-6 released by PBMC incubated with LPS and MZB)/(IL-6 released by PBMC incubated with LPS alone) × 100%. Error bars indicate the standard error of the mean (SEM). Asterisks indicate the significant suppression by MZB on IL-6 release: NS = no significant difference between IgAN and control; * $p < 0.05$, ** $p < 0.01$ in comparison with IL-6 release without MZB.

The IL-6 release by PBMC incubated with LPS and various concentrations of MZB in patients with IgAN was equivalent to that in controls as shown in figure 1 ($p > 0.05$ by Mann-Whitney U test). 10 mg/ml of MZB significantly suppressed the release of IL-6 by LPS-stimulated PBMC both in patients with IgAN (% change in IL-6: median 39.3%, range 28.1–53.5%), and in controls (% change in IL-6: median 43.2%, range 34.2–51.2%; $p < 0.05$ and $p < 0.01$, respectively, by Student-Newman-Keuls test), and a significant suppression was also observed in controls even at the lower level (0.1 mg/ml) of MZB (% change in IL-6: median 77.5%, range 65.7–85.5%, $p < 0.01$).

Discussion

Since the original description of IgAN by Berger in 1968, a tremendous research effort has failed to elucidate its pathogenesis. In the past decade, it has been postulated that numerous cytokines are elevated in IgAN, including IL-2, IL-4, IL-6, and transforming growth factor-β [10]. Among them, special attention has been paid to IL-6 because it is produced by human mesangial and tubular cells and is supposed to play an important role in mesangial cell proliferation and tubulointerstitial damage [7–9].

MZB (Bredinin®) has been shown to prevent the proliferation of lymphocytes in vitro and has been used for immunosuppression after organ transplantation [2]. It is noteworthy that MZB causes far fewer complications in patients with renal transplants than does azathioprine and does not appear to be significantly myelosuppressive, hepatotoxic or nephrotoxic [2]. Shimizu et al. [4] reported that MZB could ameliorate mesangial cell proliferation with the reduction of proteinuria in animal models of IgAN, i.e. ddY mice. Based on these facts, we and others recently reported clinical studies confirming the efficacy of this drug for the treatment of IgAN [5, 6].

There have been, however, no previous reports regarding the mode of action of MZB on IgAN. We wonder whether the MZB exerts its efficacy in IgAN via suppression of IL-6, and the present study demonstrates that this drug actually suppresses the IL-6 release by PBMC obtained from children with IgAN and control children in vitro.

However, the concentrations of MZB used in this study are much higher than those in the sera obtained from patients with IgAN taking MZB: its concentrations in the medium used in this study varied from 0.1 to 10 mg/ml, while the mean serum MZB concentration in 10 children with IgAN taking this drug was 0.62 μg/ml (unpublished data). It can be speculated that MZB is more concentrated in renal tissue than in serum, where it may locally suppress IL-6 release by macrophages/monocytes in vivo. The evidence that IL-6 mRNA is expressed in renal tissue [8] lends support to this hypothesis.

In summary, this study demonstrated for the first time that the suppression of IL-6 release by MZB plays some role in the clinical efficacy on IgAN. It seems to be worthwhile to perform in situ studies investigating the relationship between the concentrations of MZB and the degree of expression in IL-6 mRNA using animal models with IgAN, such as ddY mice.

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