Review article

Mizoribine in the treatment of pediatric-onset glomerular disease

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Background: **Mizoribine (MZR) is a selective inhibitor of inosine monophosphate dehydrogenase, a key enzyme in the pathway responsible for *de novo* synthesis of guanine nucleotides. As an immunosuppressant, MZR has been used successfully without any serious adverse effects in the treatment of renal diseases in children as well as adults. Besides its immunosuppressive effect, MZR has been reported to ameliorate tubulointerstitial fibrosis in rats via suppression of macrophage infiltration.**

Data Sources: **In this review, we summarize reported possible benefits of MZR in the treatment of pediatric-onset glomerular disease.**

Results: **We recently observed that MZR itself selectively attenuates the expression of monocyte chemoattractant protein-1 at both the mRNA and protein levels in human mesangial cells. Since MZR binds specifically to 14-3-3 proteins and heat shock protein 60, both of which are reportedly expressed in inflamed glomeruli, MZR may bind directly to inflamed glomerular cells, thereby possibly preventing progressive damage from glomerulonephritis through a suppressive effect on activated macrophages and intrinsic renal cells. Moreover, it has recently been reported that MZR directly prevents podocyte injury through correction of the intracellular energy balance and nephrin biogenesis in cultured podocyte and rat models, suggesting a direct anti-proteinuric effect of MZR.**

Conclusions: **These beneficial mechanisms of action of MZR as well as its immunosuppressive effect would warrant its use in the treatment of pediatric-onset glomerular disease. Although further studies remain to be done, we believe that MZR may be an attractive treatment of choice for children with glomerular diseases from a histologic as well as clinical standpoint.**

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Key words: **macrophage infiltration; mesangial cells; mizoribine; monocyte chemoattractant protein-1; podocytes**

Introduction

Mizoribine (MZR), a purine synthesis inhibitor, was developed in Japan about 20 years ago. The mode of action of MZR is very similar to that of mycophenolate mofetil (MMF), involving selective inhibition of inosine monophosphate dehydrogenease (IMPD) in the pathway of *de novo* purine nucleotide synthesis, which results in the suppression of T and B lymphocyte proliferation.[1,2] So far, MZR has been used successfully without serious adverse effects in the treatment of renal transplant recipients,[3] nephrotic syndrome (NS),[4,5] immunoglobulin (Ig) A nephropathy,[6,7] and lupus nephritis (LN),[8,9] mainly in Japan and East Asia. In a previous experimental setting, MZR has been reported to reduce urinary protein excretion in rat models of nephrosis induced by puromycin aminonucleoside (PAN), in addition to its immunosuppressive effect.[10] Also, it has been reported that 14-3-3 proteins, which bind to MZR, interact with glucocorticoid receptors after MZR administration, to enhance receptor transcriptional activity.[11] These laboratory observations might explain the steroid-sparing effect of MZR.

From a clinical viewpoint, MZR inhibition of IMPD is competitive, and thus different from that induced by MMF.[1,2] Therefore, the peak blood concentration of MZR required to effectively inhibit the human mixed-lymphocyte reaction is reported to be 3.0 to 6.0 μg/mL.[3] In addition, the MZR concentration required to effectively enhance steroid receptor activity via 14-3-3 proteins is

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reportedly in excess of 10 μmol/L, which corresponds to a blood MZR level of approximately 2.6 μg/mL.[11] Indeed, recent clinical reports have indicated that the efficacy of MZR may depend on the peak serum level of the drug.[12-17] Accordingly, it is now thought that a peak serum MZR level of at least 2.5-3.0 μg/mL may be needed in order to sustain long-term efficacy for the treatment of patients with glomerular diseases.[13,15] However, when using the conventional daily low-dose MZR protocol (3-4 mg/kg) in patients with NS or LN, the peak blood level of the drug usually remains at around 1.0 μg/mL,[12] which may explain the previously reported relatively mild "immunosuppressive" efficacy of MZR in clinical practice.[4,14] In this context, we and other groups have conducted trials of intermittent pulse therapy with oral MZR (up to 10 mg/kg; MZR-pulse) to attain increased peak blood levels of MZR in selective patients with NS and LN.[12,13,15-18] The results suggested that this new treatment protocol was beneficial and resulted in higher efficacy and lower toxicity than the conventional protocol.[15,17] The rationale for using MZR-pulse was as follows: 1) MZR is rapidly excreted into the urine: about 90% of the drug being completely eliminated from the circulation within about 12 hours after oral intake; therefore, accumulation of the drug is not considered to be a problem, at least under conditions of normal renal function.[1,2] Considering this point, MZR-pulse may be relatively safe; 2) Higher doses of MZR increase the area under the serum concentration-time curve (AUC) in patients; thus, the efficacy of MZR may depend on the peak serum level of the drug, which, in turn, may be closely correlated with the AUC of the drug.[19] Here, however, we do not intend to further discuss the relationship between MZR pharmacokinetics and clinical efficacy in the context of "immunosuppressive" treatment. With regard to these issues, the reader is referred to other general reviews of MZR.[1,2,19]

MZR slows the progression of histologic chronicity via suppression of macrophage infiltration

Besides its immunosuppressive effects, MZR has recently been reported to suppress the progression of histologic chronicity in selected patients with pediatric-onset IgA nephropathy and LN.[6,15,20-22] Previously, MZR has been reported to attenuate tubulointerstitial fibrosis in a dose-dependent manner in rat models of unilateral ureteral obstruction, non-insulin-dependent diabetes and peritoneal fibrosis via suppression of macrophage infiltration of the interstitium.[23-25] Interestingly, MZR has been reported to bind specifically to heat shock protein (HSP) 60, thus interfering with the chaperone activity of HSP60 *in vitro*.[26] This, in turn, may lead to suppression of the activity of α3β1-integrin, which is known to play a role in the development of interstitial fibrosis. Indeed, in a clinical setting, it has also been reported that posttreatment renal biopsy specimen from patients with severe IgA nephropathy treated with MZR showed marked attenuation of glomerular and interstitial lesions, and significantly reduced the number of activated macrophages, associated with expression of 14-3-3 proteins and HSP60, which are known to be MZR-binding proteins, in inflamed glomerular cells (typically seen as mesangial cell proliferation with cellular crescents and interstitial accumulation of leukocytes).[20] Accordingly, it is speculated that MZR may bind directly to inflamed glomerular cells and prevent progressive damage through suppression of activated macrophages and intrinsic renal cells. Therefore, MZR itself may exert a favorable effect against the progression of interstitial fibrosis in the diseased kidney. These laboratory and clinical observations suggest another beneficial mechanism of action of MZR in the treatment of renal diseases. In a recent clinical study, we confirmed the reported beneficial histologic effects of MZR, noting significant suppression of intraglomerular macrophage infiltration accompanied by significant suppression of chronicity indices following MZR treatment,[22] but, surprisingly, this was not the case with azathioprine.[27] Interestingly, MZR treatment also tended to reduce interstitial macrophage infiltration and the expression of osteopontin, known to be a chemoattractant protein for macrophages, corroborating previous experimental observations in rat models.[23] Since inflamed glomeruli reportedly express 14-3-3 proteins and HSP60,[20] MZR may directly interact with inflamed glomerular cells, because MZR is directly excreted into the urine.[28] Another recent experimental study has reported that MZR treatment reduced total glomerular macrophage accumulation with selective reduction of activated macrophages in rats with Thy-1 glomerulonephritis, whereas corticosteroid did not.[29] Taken together, the data suggest that MZR binds directly to inflamed glomerular cells, subsequently attenuating the expression of osteopontin and reducing the accumulation of activated macrophages,[22,23] thereby preventing any progressive damage by suppressing activated macrophages and intrinsic renal cells. Therefore, in addition to its immunosuppressive effects, MZR itself may slow the progression of interstitial fibrosis in the diseased kidney.

Since the proposal of a model of possible "pseudoviral" infection to explain the pathogenesis of LN,[30] we have been examining the effect of polyinosinic-polycytidylic acid (poly IC), a synthetic analogue of viral dsRNA, on toll-like receptor (TLR) 3 signaling cascades involved in "pseudoviral" infection in cultured human mesangial cells (MCs).[31-34] We found

that activation of mesangial TLR3 upregulated the expression of monocyte/macrophage chemoattractants, such as monocyte chemoattractant protein-1 (MCP-1), CC chemokine ligand (CCL) 5 (or regulated on activation, normal T-cell expression and secretion), and fractalkine [or chemokine (C-X3-C motif) ligand 1], in cultured human MCs.[31-34] Therefore, we examined the direct effects of MZR on the expression of MCP-1, CCL5 and fractalkine in cultured human MCs treated with poly IC.[35] Pretreatment of cells with MZR partially, but significantly, attenuated the expression of MCP-1 mRNA and protein, whereas the poly IC-induced expression of both CCL5 and fractalkine was not influenced by MZR treatment (Fig.). On the other hand, pretreatment of cells with tacrolimus, a calcineurin inhibitor (CNI) did not suppress the expression of MCP-1 mRNA.[35] Recently, it has been reported that MZR inhibits any increases in MCP-1 mRNA and protein dose-dependently within the range 1-100 μg/mL in thrombin-treated rat glomerular epithelial cells.[36] These experimental observations suggest that MZR, besides its immunosuppressive effect, directly inhibits MCP-1 in inflamed glomerular cells of humans as well as rats, although this theory remains preliminary. The urinary concentration of MZR reportedly reaches 400 μg/mL in pediatric patients with glomerular diseases. Even if they do not receive a high-dose of the drug,[28,37] a concentration of 100 μg/mL in the region of residual glomerular cells is not so irrelevant in a clinical setting.[35,36] Therefore, it is thought that these experimental studies further support a possible benefit of MZR in the treatment of glomerulonephritis.[23-25,29,35,36]

MZR may prevent podocyte injury

For more than 15 years, it has been reported that MZR reduces urinary protein excretion in PAN-induced rat nephrosis models.[10] However, the precise mechanism responsible remains to be determined. It has recently been reported that MZR directly prevents podocyte injury in rat PAN-induced nephropathy.[38] This protective effect is independent of IMPD, which is the target of the drug when it acts as an immunosuppressant, whereas MZR reduces PAN-induced integrin-linked kinase activation and phosphorylation of glycogen synthase kinase-3β, thereby preserving nephrin structure *in vivo* and *in vitro*, but this is not the case for MMF.[38] Moreover, it has been reported that MZR, through a mechanism that is likely dependent on inhibition of IMPD activity

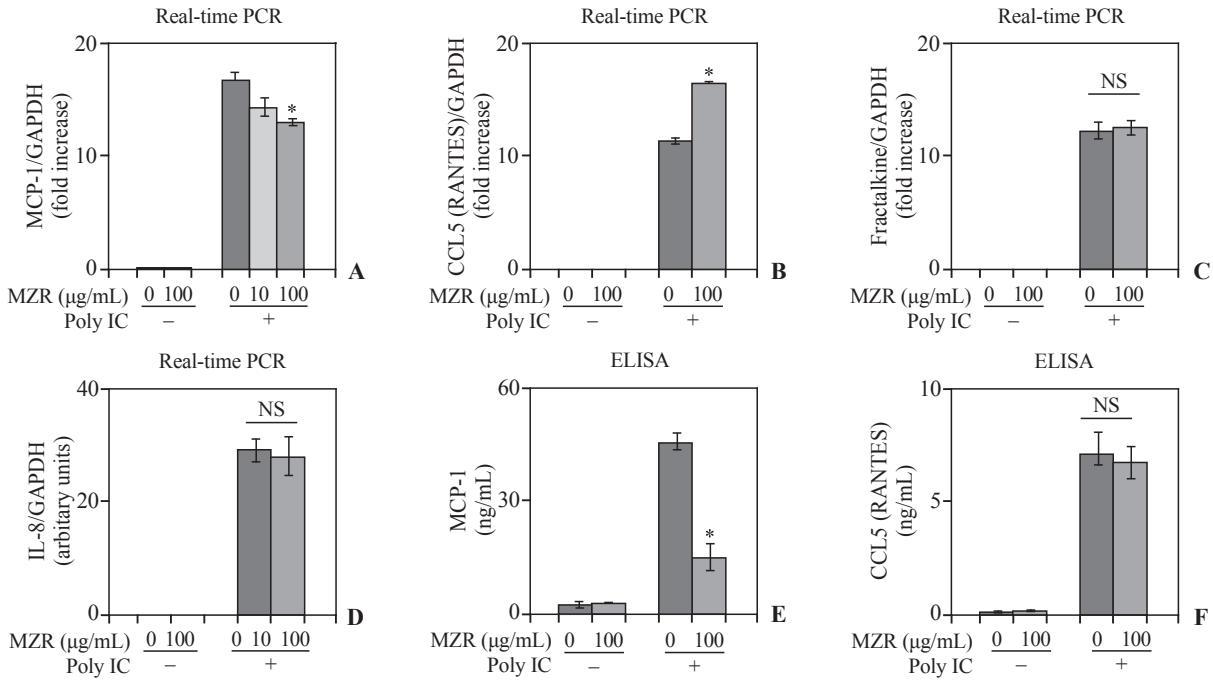

Fig. A&B: Pretreatment of cells with MZR partially, but significantly, attenuates the expression of MCP-1 mRNA, whereas the poly IC-induced mRNA expression of CCL5 (RANTES) was significantly increased; **C&D:** On the other hand, MZR did not inhibit the expression of fractalkine and IL-8 mRNA induced by poly IC; **E&F:** Cells were pretreated with 100 μg/mL MZR 1 hour before the treatment with poly IC. After incubating for 16 hours, RNA was extracted and real-time PCR was performed (n=3). Then, the cultured medium was collected and the concentration of MCP-1 and CCL5 (RANTES) in the medium was measured using an ELISA. The data were expressed as means±SD (n=3). Statistical significance was evaluated using the paired t test (This figure is quoted from our recent paper). MZR: mizoribine; MCP-1: monocyte chemoattractant protein-1; poly IC: polyinosinic-polycytidylic acid; CCL5: CC chemokine ligand 5; RANTES: regulated on activation, normal T-cell expression and secretion; IL-8: interleukin-8; GAPDH: reduced glyceraldehyde-phosphate dehydrogenase; ELISA: enzyme linked immunosorbent assay; NS: not significant. *: P<0.01.

in podocytes, restores the intracellular energy balance by increasing the levels of adenosine triphosphate and corrects the posttranslational processing of nephrin in cultured podocyte.[39] Thus, MZR may protect podocytes against loss of actin structure and cell death, leading to preservation of nephrin distribution.[38] In a clinical setting also, MZR monotherapy has been reported to exert an anti-proteinuric effect, albeit it remains temporarily, in patients with membranous glomerulopathy.[40] These experimental and clinical observations suggest a direct anti-proteinuric effect of MZR via prevention of podocyte injury, besides its immunosuppressive effects.

MZR may prevent CNI-induced nephropathy

MZR also appears to exert a beneficial protective effect against CNI nephropathy, CNI-induced intimal hyperplasia and perivascular inflammatory cell infiltration in rat models.[41,42] These studies demonstrated that MZR administration markedly attenuated the lesions of CNI nephropathy, such as macrophage accumulation and interstitial fibrosis.[42] These findings also indicate that MZR has beneficial effects on several processes, helping to prevent interstitial fibrogenesis due to CNIs-induced nephropathy. Clinically, we have recently treated children with refractory NS associated with CNI nephropathy due to previous aggressive cyclosporine treatment, in whom long-term renal function was preserved and complete remission achieved after MZR treatment.[43,44] Although the precise nature of the "renoprotective" effect of MZR in a clinical setting remains speculative, these beneficial actions of MZR would warrant its additional use in the treatment of patients with refractory NS, especially those who have undergone long-term CNI treatment.[44]

Conclusions

It is speculated that MZR may bind directly to inflamed glomerular cells and prevent progressive damage though suppression of activated macrophages and intrinsic renal cells. MZR also acts to protect podocytes through prevention of nephrin distribution. Therefore, MZR itself may exert a favorable effect against the progression of glomerular lesions. Although further detailed studies remain to be done, we believe that MZR may be an attractive treatment of choice for children with glomerular diseases from a histologic as well as clinical standpoint.

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