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Safety, tolerability and pharmacokinetics of higher-dose mizoribine in healthy male volunteers

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Aims
Mizoribine is an oral immunosuppressive agent approved in several countries for prevention of rejection in renal transplantation. Its therapeutic window is based on trough concentrations staying at ≥0.5 but <3 μg ml^{-1}. It has been postulated that as renal function returns to normal, higher doses may be needed to maintain efficacy than the current clinical dosage of 2–5 mg kg^{-1} day^{-1}. The safety, tolerability and pharmacokinetics from two clinical trials of higher-dose mizoribine treatments in healthy male volunteers are presented.

Methods
Forty-eight healthy White male nonsmokers participated in two randomized, double-blind, placebo-controlled trials: 32 in a single-dose study (3, 6, 9 and 12 mg kg^{-1}) and 16 in a multiple-dose study [6 mg kg^{-1} day^{-1} once daily for 5 days or twice daily (12 mg kg^{-1} day^{-1}) for 7 days]. Standard assessments of safety, tolerability and pharmacokinetics were performed.

Results
The safety profiles of both studies were generally unremarkable, except for elevated serum uric acid concentrations at the highest dose (12 mg kg^{-1} day^{-1}) in the multiple-dose study. Orally administered mizoribine reached peak concentrations within 2–3 h and was eliminated mostly via the kidney (65–100% of dose) with a 3-h half-life. Only the 12 mg kg^{-1} day^{-1} group achieved trough concentrations that were within the therapeutic window.

Conclusions
Based on the favourable safety profile and current pharmacokinetic information, a new starting dose in the 6–12 mg kg^{-1} day^{-1} range is recommended in the up to 3 months acute phase following transplantation, with dose reduction recommended only if the function of the transplanted kidney is impaired.

Introduction

Renal transplant is the most frequent and generally the most successful type of solid organ transplant in the world, numbering over 12 000 procedures per year in the USA and over 1500 procedures per year in the UK [1, 2]. It is estimated that more than half of all dialysis patients in the USA and in the UK are on kidney transplant lists, with the demand increasing due to aging populations in both countries, among other factors, and these transplant beneficiaries automatically become life-long recipients of immunosuppressant therapies. Current trends in immunosuppressive therapy after kidney transplantation consist of at least a triple- or a four-fold combination drug regimen [3]. However, even these aggressive

multidrug regimens can fail to prevent and control chronic rejection. Consequently, there is a continuous need to develop new immunosuppressive agents with improved efficacy and safety profiles.

Mizoribine, chemical name 4-carbamoyl-1-β-D-ribofuranosyl-imidazolium-5-olate, is an orally available immunosuppressive agent that is approved in Japan, South Korea and China for the prevention of rejection in renal transplantation. In addition, mizoribine has been approved in Japan for the treatment of lupus nephritis (1990), rheumatoid arthritis (1992) and nephrotic syndrome (1995). Mizoribine is a nucleoside of the imidazole class of compounds, inhibiting the proliferation of lymphocytes by blockade of nucleic acid synthesis. It was originally isolated as a substance having weak antimicrobial activity against *Candida albicans* [4] and was subsequently found to inhibit both humoral and cellular immunity by selectively inhibiting the proliferation of lymphocytes via inhibition of *de novo* purine biosynthesis [5]. In contrast to other immunosuppressive agents (e.g. azathioprine), mizoribine has been shown in animal experiments to lack oncogenicity and has shown clinically a low incidence of severe adverse drug reactions (such as myelosuppression and hepatotoxicity), making it useful in long-term immunosuppressive therapy [6]. In addition, its low incidence of adverse events at current clinical doses potentially makes high-dose mizoribine an attractive alternative immunosuppressive agent for rescue treatment of ongoing acute rejections [7].

Mizoribine has been shown to be safe and well tolerated in renal transplant patients at doses of up to 5 mg kg^{-1} day^{-1}. To date, all pharmacokinetic (PK) information about mizoribine comes from two studies in renal transplant patients: a single-dose study [8] and a multiple-dose study [9]. In both studies a positive correlation existed between peak serum concentrations, occurring between 2 and 4 h postdose, and oral dose. Neither of the patient studies evaluated the bioavailability (F) of mizoribine; however, based on urinary excretion data in renal transplant recipients, a wide range of oral bioavailability of mizoribine has been observed (12–81%) [10].

In both the single- and multiple-dose studies, mizoribine did not appear to be hepatically metabolized and most of the oral dose was excreted unchanged in the urine. Total body clearance of mizoribine approximated creatinine clearance. The reported elimination half-life ($t_{1/2}$) ranged from 2 to 18 h, depending on the degree of renal impairment, and therefore, as mizoribine is directly excreted through the kidneys, its dose has to be adjusted as the renal function of the patient improves.

Both the efficacy and safety of mizoribine have been correlated to its trough concentrations. *In vitro* studies have shown that plasma tough concentrations of 0.5 μg ml^{-1} inhibit T-lymphocyte proliferation by 50% [11]; thus, a trough of 0.5 μg ml^{-1} is considered to result in sufficient inhibitory effect on organ rejection in the acute phase of 3 months following transplantation. Further, it has been reported that at trough concentrations of ≥3 μg ml^{-1}, adverse events such as myelosuppression, infectious disease and alopecia manifest.

In the single-dose [8] and multiple-dose [9] PK studies, the renal transplant patients had decreased renal function and the 3–5 mg kg^{-1} dose range resulted in trough concentrations of 0.5 μg ml^{-1}, and thus exhibited a sufficient inhibitory effect on rejection. However, as renal function returned to normal, the current 5 mg kg^{-1} day^{-1} upper limit of the dose range was suspected not to be sufficient to maintain an acceptable inhibitory effect on rejection. Doses of up to 10.2 mg kg^{-1} day^{-1} have been given to a small number of patients, with no apparent serious adverse reactions [12]; however, no formal assessments of the safety and pharmacokinetics of higher-dose mizoribine in subjects with normal renal function have been performed. This report summarizes the safety, tolerability and PK results from two clinical trials, one single dose and one multiple dose, of higher-dose mizoribine treatment in healthy male volunteers. The target maximum 12 mg kg^{-1} day^{-1} dose, administered as twice-daily 6 mg kg^{-1} doses in the multiple-dose study, was expected to be the lowest daily dose to result in a sufficient inhibitory effect on organ rejection in patients with normal renal function, assuming that the pharmacokinetics of mizoribine remained linear and time independent at these higher doses.

Methods

Subjects

In total, 48 healthy White male nonsmokers participated in the two trials. Thirty-two subjects, aged 18–45 years (mean 27 years), weighing 59–93 kg (mean 78 kg) and Cr_{CL} range 101.9–164.1 ml min^{-1}, participated in the single-dose study; and 16 subjects, aged 18–44 years (mean 25 years), weighing 54–91 kg (mean 74 kg) and Cr_{CL} range 80.3–197.9 ml min^{-1}, participated in the multiple-dose study. All 32 subjects completed the single-dose study. One subject randomized to 12 mg kg^{-1} day^{-1} treatment in the multiple-dose study withdrew consent for study participation due to personal reasons and the remaining 15 subjects completed the multiple-dose study. Both studies excluded subjects with any history of alcohol or drug abuse within 2 years prior to the study, an abnormal diet or substantial changes in

eating habits within 30 days prior to study initiation, hypersensitivity or idiosyncratic reaction to the study drug or related compounds, or clinically significant abnormal findings on physical examination, 12-lead ECG, medical history, or clinical laboratory results during screening. Furthermore, subjects were excluded if they had treatment with any known enzyme-altering agents (barbiturates, phenothiazines, cimetidine, etc.) within 30 days prior to or during the study, or had taken any prescription medication within 14 days prior to or during the study, or any over-the-counter medication within 7 days prior to or during the study. Subjects were to have no foods or beverages containing alcohol or caffeine/xanthine within 48 h prior to or during the period of confinement. Grapefruit was prohibited for 10 days prior to and during the period of confinement.

Both studies were conducted at MDS Pharma Services in Belfast, Northern Ireland, in accordance with the latest version of the World Medical Association Declaration of Helsinki and ICH Guideline for Good Clinical Practice (GCP). The Queen's University Research Ethics Committee (REC) reviewed the protocols and informed consent forms prior to initiation of each study. The Queen's University Belfast REC operations are in compliance with Section 56 of Title 21 of the Code of Federal Regulations (CFR).

Study design, rationale, and treatments administered

Both studies were placebo controlled, with drug dosing based on total body weight. Using 50-mg tablets, subjects were dosed within ±20% of the assigned dose level. Subjects randomized to placebo received an equivalent number of placebo tablets to those receiving active drug. Each dose of study treatment was administered with 240 ml of room temperature tap water. Subjects were not permitted to lie down for the first 4 h following administration of the drug to assure proper stomach emptying.

The single-dose study had a randomized, double-blind, placebo-controlled, ascending (3, 6, 9 and 12 mg kg^{-1}) single-dose design. The 32 healthy male subjects were enrolled in four dose levels of eight subjects: six received mizoribine and two received placebo. Doses were administered after an overnight fast, with standardized meals administered starting at 4 h postdose. Confinement to the clinic lasted until the 24 h PK blood sample was drawn and safety assessments were completed. Subjects were required to return to the clinic approximately 7 days postdose for post-study follow-up safety assessments. Dose escalation was based upon safety assessment of vital signs, 12-lead ECG, adverse events (AEs) and clinical laboratory data. Due to a potential myelosuppressive action of mizoribine, the haematological profile (leucocytes, platelets, etc.) of the subjects was monitored before and after dosing. Particular attention was also paid to renal function before dosing.

The multiple-dose study had a randomized, double-blind, placebo-controlled, ascending multiple-dose design with two dose levels of mizoribine investigated in two groups of eight subjects: six received mizoribine and two received placebo. In dose group 1, each subject received a once-daily administration of 6 mg kg^{-1} mizoribine or placebo, after an overnight fast, for 5 days. In dose group 2, each subject received 6 mg kg^{-1} mizoribine twice daily (12 mg kg^{-1} day^{-1}) or placebo twice daily, after an overnight fast, for 7 days. Fasting was not required prior to the evening dose. Dose escalation was based upon safety assessment of vital signs, 12-lead ECG, adverse events and clinical laboratory data.

Safety and tolerability assessments

Subjects who participated in the studies met all eligibility criteria, provided medical and surgical histories and completed the study screening assessments (clinical laboratory measurements, vital sign and ECG measurements and physical examination). During the studies, the safety of mizoribine was evaluated by means of clinical laboratory evaluations, physical examinations, vital sign and ECG measurements and AE reports.

Pharmacokinetic analyses

Serum and urine samples were analysed for mizoribine using a validated LC/MS/MS method by the Pharmacokinetics and Bioanalysis Centre of Shin Nippon Biomedical Laboratories, Ltd [13]. The lower limit of quantification (LLOQ) of the assay was 0.0250 μg ml^{-1} for both serum and urine, and all concentrations below the LLOQ were treated as zero for the purpose of PK analysis and presentation of descriptive statistics. All serum PK parameters were calculated from the concentration-time data following each dosing group by model-independent methods using WinNonlin Professional, Version 4.0 (Mountain View, CA, USA) and SAS® (Release Version 8.2 for Open VMS; SAS Institute Inc., Cary, NC, USA). All urinary PK parameters were calculated using SAS®.

Single-dose study

Blood samples were collected for mizoribine analysis at predose (hour 0) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h postdose. The PK parameters evaluated included C_{max} (maximum observed drug concentration); t_{max} (time to

reach C_{max}); $t_{1/2}$ (apparent terminal half-life; calculated as $\ln(2)/k_{el}$, where k_{el} is the apparent terminal elimination rate constant calculated by linear regression of the terminal linear portion of the log concentration *vs.* time curve); AUC_{0-t} (area under the drug concentration–time curve calculated using linear trapezoidal summation from time zero to time t, where t is the time of the last measurable concentration [Ct]); $AUC_{0-\infty}$ (area under the drug concentration–time curve from time zero to infinity, calculated as $AUC_{0-\infty} = AUC_{0-t} + Ct/k_{el}$); AUCR (ratio of AUC_{0-t} to $AUC_{0-\infty}$); CL/F [oral clearance, calculated as (Drug dose)/$AUC_{0-\infty}$)]; and V_z/F (apparent volume of distribution following oral administration, calculated as $CL/F/k_{el}$).

Urine samples for mizoribine analysis were collected prior to dosing (–8–0 h), 0–8 h and 8–24 h postdose. For each collection interval, mizoribine concentration, amount and percentage dose excreted were reported. Overall percentage dose excreted (total % dose) and renal clearance (CL_R), calculated as cumulative amount/AUC_{0-t}, were estimated from the 24-h urinary excretion data.

Multiple-dose study

For dose group 1 (6 mg kg^{-1}, once daily, days 1–5), blood samples were collected for mizoribine analysis at predose (hour 0) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24 (day 2 predose), 48 (day 3 predose), 72 h (day 4 predose) and at the following time points on day 5: predose (hour 0 of day 5 or hour 96 of day 1) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h postdose. For dose group 2 (6 mg kg^{-1} twice daily, days 1–7), blood samples were collected during each study period at predose (hour 0) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24 (day 2, a.m. predose), 48 (day 3, a.m. predose), 72 (day 4, a.m. predose), 96 (day 5, a.m. predose), 120 h (day 6, a.m. predose) and at the following time points on day 7: a.m., predose (hour 0 of day 7 or hour 144 of day 1) and at 0.5, 1, 2, 3, 4, 6, 8 and 12 h postdose. Urine was not collected for mizoribine analysis in this study.

The following PK parameters were calculated for both groups on day 1: C_{max}, t_{max}, AUC_{0-t}, $AUC_{0-\infty}$, AUCτ (area under the drug concentration–time curve during a dosing interval), $t_{1/2}$, k_{el}, CL/F and V_z/F. On day 5 (dose group 1) or day 7 (dose group 2), the following PK parameters were calculated: C_{max}, t_{max}, AUCτ, $t_{1/2}$, k_{el} and CL_{ss}/F [apparent oral clearance at steady state, calculated as (Drug dose)/AUCτ]. In addition to the above, accumulation ratio (AUCτ day 5 or 7/AUCτ day 1) and time-related linearity within the dose level (AUCτ day 5 or 7/$AUC_{0-\infty}$ day 1) were also calculated.

Immunoglobulin and lymphocyte density assessments

Blood samples were collected for the analysis of immunoglobulins (IgG, IgM and IgA) and lymphocyte density (CD4+ and CD8+) for both studies. For the single-dose study, blood samples were collected at 24 h predose (baseline) and 6 h postdose. For the multiple-dose study, blood samples were collected at 24 h predose (baseline) and at 6, 78 (6 h post day 4 a.m. dose) and 102 (dose group 1, 6 h post day 5 dose) or 150 h (dose group 2, 6 h post day 7 a.m. dose). Values for the 6-h postdose assessment were presented with and without adjustment for baseline. No additional parameters were determined from the immunoglobulin biosynthesis and lymphocyte differentiation data.

Statistical analysis

Sample size for both studies was based on practical considerations rather than any power calculations. A sample size of eight subjects per group, with six receiving mizoribine and two receiving placebo, was deemed adequate to meet the objectives of each study. The double-blinded study design was selected to minimize subject and investigator bias.

Safety data, including clinical laboratory evaluations, physical examination, vital signs and 12-lead ECG assessments, were summarized by treatment group and time point. AEs were coded using MedDRA version 7.0 and summarized by treatment group.

For the single-dose study, dose proportionality of mizoribine doses was assessed for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ using a power model [14]. Using this model, the slope, standard error and the 95% confidence intervals (CI) were calculated for each parameter. The existence of dose proportionality could not be rejected if the 95% CI included 1.

For the multiple-dose study, the attainment of steady state was evaluated statistically by regressing the days 3, 4 and 5 (dose group 1) and days 5, 6 and 7 (dose group 2) trough concentrations for each dose level over time. The presence of steady state could not be rejected if the 95% CI of the slope included zero. Accumulation ratio and linearity within each dose level were assessed using descriptive statistics.

The effect of mizoribine dose level and frequency of dosing (twice daily *vs.* once daily) on immunoglobulin biosynthesis and lymphocyte differentiation was evaluated using ANOVA. For the single-dose study, mizoribine 3, 6, 9 and 12 mg kg^{-1} dose levels were compared with placebo. For the multiple-dose study, dose frequency (once daily or twice daily) was compared with placebo at –24 h predose and at 6 h postdose on days 1, 4 and the last day of the study (day 5 or 7). In those cases where

the overall treatment effect was statistically significant at 0.05 probability level, each mizorbine dose was compared with placebo. Dunnet's test was used to adjust for multiple comparisons.

Results

Safety and tolerability

Mizoribine administered in single oral doses of 3, 6, 9 and 12 mg kg^{-1} or in multiple oral doses of 6 mg kg^{-1} once daily for 5 days or 6 mg kg^{-1} twice daily for 7 days appeared to be safe and well tolerated by the two groups of healthy male volunteers studied.

The safety profile of the single-dose study was generally unremarkable. There were no serious AEs reported, no subject discontinued the study due to an AE and only three (9%) of the 32 subjects dosed (24 with mizoribine and eight with placebo) experienced a treatment-emergent AE. All treatment-emergent AEs in this study were reported by one subject each (3%) and included single mild episodes of arthralgia (following placebo), epistaxis (following mizoribine 3 mg kg^{-1}) and pharyngolaryngeal pain (following mizoribine 3 mg kg^{-1}). The investigator considered none of these AEs to be related to study drug and all AE episodes resolved by the end of the study.

The safety profile of the multiple-dose study was also generally unremarkable. A single serious AE was reported (situational depression considered unlikely to be related to study treatment) and no subject discontinued the study due to an AE. Of the 16 subjects dosed in the study (12 with mizoribine and four with placebo), eight (50%) experienced a total of 10 treatment-emergent AEs; the investigator considered two of the 10 AEs (eye pain and pharyngolaryngeal pain) to be possibly related to the study treatment. A similar number of subjects reported AEs following both placebo (three subjects) and mizoribine 6 mg kg^{-1} administered twice daily (four subjects) and only one subject reported an AE following mizoribine 6 mg kg^{-1} administered once daily. Excluding a moderate episode of influenza-like illness, all AEs were mild in severity and resolved by the end of the study.

There were no treatment-related trends observed in the leucocyte and liver function test findings for the two studies. There were also no notable trends or clinically relevant abnormalities observed in the clinical laboratory values, vital sign and ECG measurements, or physical examination parameters, except for elevation in serum uric acid. While the mean serum uric acid values remained within reference range at all assessed time points in both studies, the mean changes from baseline to the initial postdose assessment appeared to be dose related in both studies and the magnitude of these mean changes was particularly marked for the highest dose level (12 mg kg^{-1} day^{-1}).

At the 24-h assessment following single-dose administration, serum uric acid mean values decreased from baseline for the placebo (−23.25 μmol l^{-1}) and 3 mg kg^{-1} (−19.84 μmol l^{-1}) groups, but increased from baseline for the 6 mg kg^{-1} (+18.17 μmol l^{-1}), 9 mg kg^{-1} (+25.67 μmol l^{-1}) and 12 mg kg^{-1} (+36.84 mg kg^{-1}) groups. There did not appear to be a treatment-related trend in the mean serum uric acid changes from baseline at the subsequent assessment (hour 120) in the single-dose study.

At the 72-h assessment in the multiple-dose study, serum uric acid mean values increased for all treatment groups in a dose-dependent manner: +2.00 μmol l^{-1} following placebo administration, +11.00 μmol l^{-1} following 6 mg kg^{-1} day^{-1} administration and +128.00 μmol l^{-1} following 12 mg kg^{-1} day^{-1} administration. Mean serum uric acid also markedly increased from baseline (+99.40 μmol l^{-1}) at hour 168 following 12 mg kg^{-1} day^{-1} administration. All other serum uric acid mean changes from baseline in the multiple-dose study were unremarkable.

This elevation in serum uric acid following mizoribine administration in the single-dose and multiple-dose studies is consistent with previous clinical observations with renal transplant patients [12].

Pharmacokinetics

Single-dose study The single-dose exposure (C_{max} and AUC) of mizoribine increased relatively proportionately with dose within the 3–12 mg kg^{-1} dose range investigated (Figure 1). As illustrated in Figure 1, increases in effect were in proportion to increases in dose for both C_{max} and AUC. This observation is supported by the statistical results. The 95% CI for slope calculated using the power model included the value of 1, indicating that the existence of dose proportionality could not be rejected. Also, consistent with dose-proportional PK, parameters which are generally considered dose independent did not show any change with increasing doses: median t_{max} ranged from 2 to 2.5 h for all dose levels, mean $t_{1/2}$ stayed at slightly below 3 h and CL/F and V_z/F remained at approximately 190 ml h^{-1} kg^{-1} and 780 ml kg^{-1}, respectively (Table 1).

Most of the oral dose was excreted unchanged in urine in the first 24 h (mean 65% following the 3 mg kg^{-1} dose to 104% following the 6 mg kg^{-1} dose), and renal clearance was also dose independent, alhough highly variable, ranging from 122 ml h^{-1} kg^{-1} following the

3 mg kg^{-1} dose to 176 ml h^{-1} kg^{-1} following the 6 mg kg^{-1} dose. The slightly higher than 100% mean excretion following the 6 mg kg^{-1} dose was most likely an artefact of urinary assay precision, considering that renal clearance at that dose level was reported as 176 ml h^{-1} kg^{-1}, which was more than the estimated apparent oral clearance (CL/*F*) value of 162 ml h^{-1} kg^{-1} at that dose. Physiologically, renal clearance can at a maximum account for 100% of total body clearance, and only for a drug that is exclusively excreted via the kidney and has 100% oral bioavailability (*F*), which mizoribine does not (reported *F* ranging from 12% to 81% [10]).

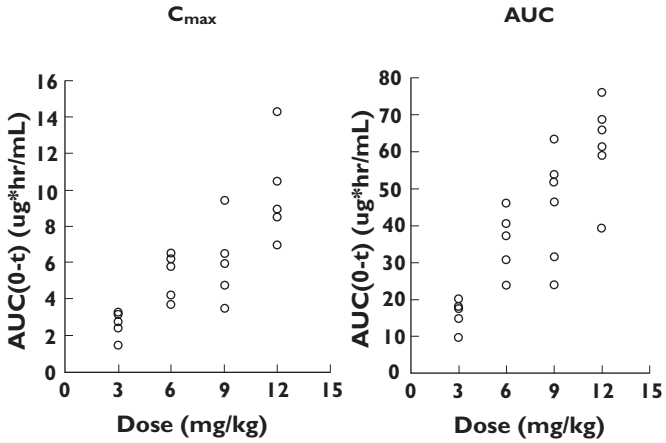

Figure 1
Individual pharmacokinetic parameters following single mizoribine doses. Individual value (○)

However, all urine samples were re-assayed and confirmed, as were the total urine volumes collected in the clinic. For the remaining three dose levels, renal clearance of mizoribine represented approximately 71–78% of CL/*F*.

Multiple-dose study The therapeutic window of mizoribine, at least in the acute phase following transplantation, has been defined in terms of the trough concentrations remaining within the 0.5–3 μg ml^{-1} range, and neither the once-daily nor the twice-daily dosing regimens resulted in trough concentrations that were above the 3 μg ml^{-1} upper limit of the therapeutic window (Figure 2). In fact, following the first dose of the twice-daily dosing regimen, the mean trough concentration at 12 h post dose was 0.469 μg ml^{-1} (range 0.155–0.655 μg ml^{-1}), indicating that in subjects with normal renal function the 12 mg kg^{-1} day^{-1} dose given as 6 mg kg^{-1} twice daily achieves target efficacy concentrations by the first dose. At steady state (day 7), the trough concentrations were at a mean of 0.834 μg ml^{-1} (range 0.307–1.181 μg ml^{-1}), which was well within the therapeutic window. However, the once-daily dosing regimen did not maintain the trough concentrations within the therapeutic window. The mean 24-h postdose concentrations after the first dose was only 0.050 μg ml^{-1} (range 0.025–0.116 μg ml^{-1}) and troughs remained relatively unchanged through all the doses (Figure 2), with the trough at predose of day 5 at 0.045 μg ml^{-1} (range 0.026–0.108 μg ml^{-1}). The statistical results of the steady-state assessment indicated that steady state could

Table 1
Summary of the single rising dose pharmacokinetic parameters (mean ± SD) of mizoribine in serum and urine (total $N = 24$; six per dose level)

Parameter	3 mg kg^{-1}	6 mg kg^{-1}	9 mg kg^{-1}	12 mg kg^{-1}
Serum				
C_{max} (μg ml^{-1})	2.7 ± 0.7	5.5 ± 1.2	6.6 ± 2.4	9.6 ± 2.5
t_{max}* (h)	2.0 (2.0–3.0)	2.5 (2.0–3.0)	2.1 ± (2.0–3.0)	2.0 ± (2.0–2.1)
AUC_{0-t} (μg h^{-1} ml^{-1})	16.7 ± 4.0	35.9 ± 7.7	45.1 ± 14.7	61.6 ± 12.5
$AUC_{0-\infty}$ (μg h^{-1} ml^{-1})	18.1 ± 3.6	38.3 ± 7.1	46.4 ± 14.0	61.9 ± 12.5
AUCR	0.92 ± 0.06	0.94 ± 0.04	0.96 ± 0.04	1.0 ± 0.002
k_{el} (l h^{-1})	0.26 ± 0.06	0.26 ± 0.03	0.25 ± 0.03	0.2 ± 0.03
$t_{1/2}$ (h)	2.9 ± 0.9	2.7 ± 0.3	2.8 ± 0.3	2.9 ± 0.3
CL/*F* (ml h^{-1} kg^{-1})	173 ± 41	162 ± 36	213 ± 77	202 ± 52
V_d/F (ml kg^{-1})	753 ± 448	637 ± 199	858 ± 304	860 ± 273
Urine				
Total % dose	65 ± 22	104 ± 27	79 ± 35	78 ± 15
CL_R (ml h^{-1} kg^{-1})	122 ± 37	176 ± 30	157 ± 42	157 ± 21

**Median and range for* t_{max}.

Figure 2
Individual trough concentrations of mizoribine following 6 mg kg^{-1} once-daily and twice-daily doses. 1 (-▲-), 2 (-◆-), 3 (—▲—), 4 (-■-), 5(—◆—), 8 (—■—)

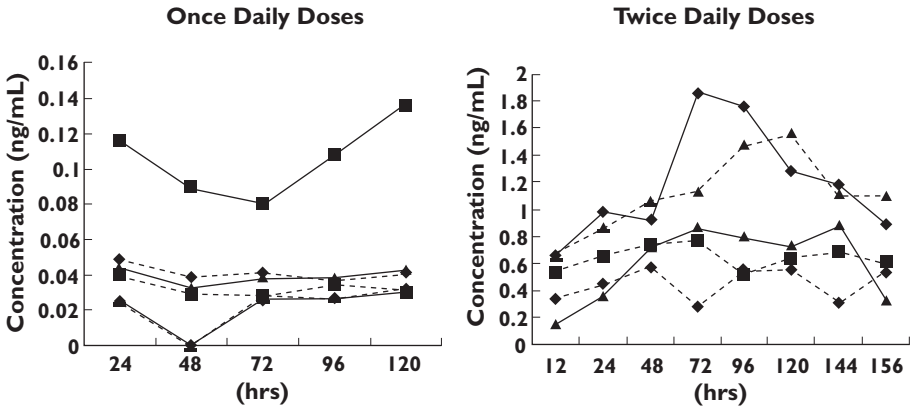

Table 2
Summary of the single- and multiple-dose pharmacokinetic parameters (mean ± SD) of mizoribine in serum (total $N = 12$, six for once-daily and six for twice-daily dosing, except where noted)

Parameter	Day 1: 6 mg kg^{-1} once daily	Day 1: 6 mg kg^{-1} twice daily
C_{max} (μg ml^{-1})	4.1 ± 1.1	4.0 ± 1.9
t_{max}* (h)	3.0 (2.0–4.0)	2.0 (2.0–3.0)
AUC_{0-t} (μg h^{-1} ml^{-1})	31.3 ± 8.5	22.1 ± 11.0
$AUC_{0-\infty}$ (μg h^{-1} ml^{-1})	31.5 ± 8.7	25.8 ± 12.1 ($N = 5$)
AUCτ (μg h^{-1} ml^{-1})	31.3 ± 8.6	22.1 ± 11.0
k_{el} (l h^{-1})	0.3 ± 0.02	0.3 ± 0.05 ($N = 5$)
$t_{1/2}$ (h)	3.0 ± 0.3	3.0 ± 0.7 ($N = 5$)
CL/F (ml h^{-1} kg^{-1})	203 ± 57	283 ± 142 ($N = 5$)
V_z/F (ml kg^{-1})	883 ± 230	1174 ± 546 ($N = 5$)

Parameter	Day 5: 6 mg kg^{-1} once daily	Day 7: 6 mg kg^{-1} twice daily
C_{max} (μg ml^{-1})	4.7 ± 0.9	4.6 ± 1.6 ($N = 5$)
t_{max}* (h)	2.0 (2.0–4.0)	3.0 (2.0–3.0) ($N = 5$)
AUCτ (μg h^{-1} ml^{-1})	35.5 ± 8.2	31.2 ± 11.0 ($N = 5$)
k_{el} (l h^{-1})	0.2 ± 0.02	0.2 ± 0.04 ($N = 5$)
$t_{1/2}$ (h)	3.0 ± 0.3	3.1 ± 0.6 ($N = 5$)
CL_{ss}/F (ml h^{-1} kg^{-1})	176 ± 34	210 ± 65 ($N = 5$)
Accumulation ratio	1.15 ± 0.14	1.42 ± 0.40 ($N = 5$)
Dose linearity	1.15 ± 0.14	1.31 ± 0.41 ($N = 5$)

**Median and range for t_{max}.*

not be rejected for either the once-daily or the twice-daily treatments.

For any drug, concentrations increase until steady state is achieved, and the degree of accumulation is generally evaluated based on relative ratios of AUC within a dosing interval following the first and last dose of each dosing regimen. On this study, the once-daily dosing resulted in an approximate 15% accumulation, whereas the twice-daily dosing resulted in an approximate 42% accumulation (Table 2). The extent of accumulation of mizoribine at steady state was consistent with that expected from once- or twice-daily administration of an orally administered drug that reaches C_{max} within 2–3 h, followed by an elimination phase with a median 3-h half-life. Although the 15% accumulation following the once-daily dosing regimen was relatively small, it could have been expected to be even smaller given that the once-daily dosing regimen is essentially

Table 3
Change from baseline (mean ± SD) in immunoglobulin concentrations and lymphocyte densities at 6 h following single mizoribine doses ($N = 32$, six per dose level)

Pharmacodynamic parameters	3 mg kg^{-1}	6 mg kg^{-1}	9 mg kg^{-1}	12 mg kg^{-1}	Placebo
IgG (mg dl^{-1})	−154.3 ± 70.0	74.3 ± 150.9	−18.0 ± 57.7	0.2 ± 40.9	−102.4 ± 147.3
IgM (mg dl^{-1})	−10.2 ± 34.8	111.5 ± 122.8	5.8 ± 5.1	−2.3 ± 7.3	−38.3 ± 58.9
IgA (mg dl^{-1})	35.7 ± 53.2	38.8 ± 77.3	4.3 ± 7.8	0.0 ± 9.4	−53.6 ± 74.9
CD4+ (cells µl^{-1})	−148.2 ± 240.0	−34.2 ± 117.2	−84.2 ± 249.1	−35.3 ± 289.7	−44.4 ± 220.4
CD8+ (cells µl^{-1})	−119.5 ± 95.2	−65.7 ± 39.2	−201.5 ± 320.8	−16.0 ± 83.2	−61.4 ± 96.0

equivalent to seven post-t_{max} mizoribine elimination half-lives. However, the actual differences in mean AUCτ values between day 1 and day 5 were approximately one-half standard deviation of the mean of this parameter on each study day; consequently, it is difficult to differentiate accurately between true accumulation and variability in this parameter. For twice-daily dosing, the degree of accumulation was greater than that of the once-daily dosing and is consistent with the shorter dosing interval (three post-t_{max} mizoribine elimination half-lives). Surprisingly, the mean C_{max} was similar between the once-daily and twice-daily dosing groups, and this was true on both day 1 and the last study day. This was unexpected given the increases in the trough values and the larger accumulation reported for the twice-daily dosing; however, it may be partially due to the large variability in individual C_{max} values, particularly following the twice-daily dosing.

Evaluation of any time-dependent changes in the PK of mizoribine was based on the comparison of linearity between AUCτ following the last dose and $AUC_{0-\infty}$ following the first dose of each dosing regimen. The results have shown that there were no dramatic differences in exposure to mizoribine following the first and last doses of each dosing regimen. For the once-daily dosing regimen, the AUC on day 5 was 15% higher than on day 1, a difference that is negligible in view of the rather small sample size and relatively large variability in AUC values on both study days. Notably, the mean values of AUCτ on day 5 compared with $AUC_{0-\infty}$ on day 1 were within one-half standard deviation of each other. The differences in linearity following the twice-daily dosing regimen were somewhat larger, at approximately 31%. However, the day 1 $AUC_{0-\infty}$ values were calculable in only five of the six subjects, and in one of the five subjects the AUC estimates were unusually low compared with other subjects in that treatment group, resulting in a lowering of the overall mean. By day 7, this subject's AUC values were more in line with those of the other subjects and, consequently, his dose linearity estimate was >2, while that of the other subjects was much closer to 1. Exclusion of this subject's data from the dose linearity estimate reduced the dose linearity value for the remaining subjects to a mean of 1.14, and a 14% difference can be considered indicative of linear pharmacokinetics of mizoribine, given the intersubject variability in AUC estimates on any given study day. Consistent with a lack of time dependency in PK, the median t_{max} (2–3 h) and the mean $t_{1/2}$ (approximately 3 h) remained unchanged by repeated dosing of mizoribine.

Immunoglobulin concentrations and lymphocyte density assessments

Overall, following single doses of mizoribine, the mean baseline-adjusted IgG, IgM and IgA concentrations and CD4+ and CD8+ densities remained relatively unchanged compared with baseline and with placebo (Table 3). Statistically significant differences in IgG, IgM and IgA were observed following the 6 mg kg^{-1} dose compared with placebo, and in IgA following the 3 mg kg^{-1} dose compared with placebo (Dunnett's adjusted p-values <0.05). However, there were no statistically significant differences between any of these assessments and placebo at the higher dose levels (9 mg kg^{-1} and 12 mg kg^{-1}).

For the multiple-dose study, there were no statistically significant differences in blood IgG, IgM or IgA immunoglobulin concentrations or CD4+ and CD8+ lymphocyte densities compared with placebo on any of the study days evaluated following mizoribine doses of up to 12 mg kg^{-1} daily for 7 days.

Discussion

In vitro studies have linked both the efficacy and the toxicity of mizoribine to its trough concentrations. In the

acute phase following transplantation, inhibitory effects on organ rejection are maintained for as long as trough concentrations do not drop below 0.5 μg ml^{-1}, while safety becomes a concern at trough concentrations >3.0 μg ml^{-1}. Historically, the current clinical dosage range of 2–5 mg kg^{-1} day^{-1} has been shown to be both safe and effective in early acute stages following renal transplantation; however, as calcineurin inhibitors quickly improve renal function, it is suspected that dosage adjustments beyond the 5 mg kg^{-1} day^{-1} upper limit may be necessary to maintain efficacy.

The presented data from the single- and multiple-dose PK studies have confirmed that at doses up to 12 mg kg^{-1} day^{-1} mizoribine is almost predominantly eliminated renally; that peak exposure and overall exposure increase proportionately with dose and do not show any substantial evidence of time dependency; and, most importantly, that at the 12 mg kg^{-1} day^{-1} dose level, administered in two daily 6 mg kg^{-1} doses, the steady-state trough concentrations stay within the therapeutic window of ≥0.5 μg ml^{-1} but <3.0 μg ml^{-1} in subjects with normal renal function (Cr_{CL} range across the two studies from 80.3 ml min^{-1} to 197.9 ml min^{-1}). Furthermore, following these twice-daily doses, mean trough concentrations were at the minimum effective concentration practically by the first dose (mean at 12 h of 0.469 μg ml^{-1}). Following 6 mg kg^{-1} day^{-1} daily doses, the trough concentrations were at levels substantially below the 0.5 μg ml^{-1} minimum effective concentration and consequently the current clinically used dosing range of 2–5 mg kg^{-1} day^{-1} is not sufficient to maintain mizoribine at the minimum effective concentration in subjects with normal renal function.

The two studies have also shown that mizoribine pharmacokinetics are very suitable for its intended use as an oral immunosuppressant. It is readily orally available and rapidly absorbed, with detectable concentrations reached within 0.5 h postdose and peak concentrations achieved within 2–3 h, followed by a relatively monoexponential decline with a 3-h elimination half-life. These t_{max} and $t_{1/2}$ values were consistent between the two studies, and thus were dose and frequency of dose (once daily or twice daily) independent up to the highest dose investigated (12 mg kg^{-1} day^{-1}). Given that the healthy male participants in both studies had normal kidney function, the observed 3-h terminal half-life was consistent with the lower range of the previously reported elimination half-life of 2–18 h, which was found to depend on the degree of renal impairment [10].

The high renal excretion of mizoribine (more than 65% of dose excreted intact in urine more than 70% of CL/F to CL_R) indicates that dosage adjustments must be made frequently as renal function returns to normal after transplant, while the ultimately short half-life may necessitate daily doses up to 6 mg kg^{-1} twice daily (12 mg kg^{-1} day^{-1}) to maintain trough concentrations in the therapeutic window. Yet the short half-life also makes this drug attractive in terms of managing harmful events and the ability to reach target concentrations quickly. In particular, a relatively short half-life is very useful for management of paediatric and elderly patients, as well as for the management of ongoing acute rejections for which mizoribine's usefulness has been indicated [7], and where rapid titration of drug concentrations is critical. In addition, mizoribine is expected to be useful as therapy following nonrenal transplantations, in part due to its extensive renal excretion. For example, in recent years it has been used following liver transplantation in Japan, and in fact its use for liver transplantation is increasing, since its excretion is not dependent on liver function, indicating that dose adjustments should be less of an issue with liver than with renal transplantations.

The lack of apparent or consistent change in the mean IgG, IgM and IgA concentrations or CD4+ and CD8+ densities compared with baseline and compared with placebo in both studies was not unexpected, given that no immunosuppressive effects of mizoribine monotherapy were observed in subacute toxicity studies at doses up to 20 mg kg^{-1} day^{-1} in beagle dogs [6]. In clinical trials, immunosuppression has been seen only when mizoribine was administered in combination with other immunosuppressants. Even then, less leukopenia was observed in transplant patients receiving mizoribine combination therapy than in those receiving other immunosuppressant combination therapies. In the present Phase I studies, mizoribine did not produce immunosuppression over the dose- and time-range tested.

Since mizoribine trough concentrations remained well below 3.0 μg ml^{-1}, the safety profiles of both the single- and multiple-dose studies were generally unremarkable. Overall, doses of up to 12 mg kg^{-1} day^{-1} appeared to be well tolerated by the two groups of healthy male volunteers, except for elevated serum uric acid concentrations at the highest dose (12 mg kg^{-1} day^{-1}) in the multiple-dose study. No other notable trends or clinically relevant abnormalities were observed in the clinical laboratory values, vital sign and ECG measurements, or physical examination parameters. Overall, the majority of AEs experienced in both studies were mild and considered unrelated to the study drug.

In conclusion, mizoribine is an oral immunosuppressant with a well-documented low incidence of AEs and its excellent safety profile was maintained through

the current high-dose single- and multiple-dose administrations of up to 12 mg kg^{-1} day^{-1} to healthy male volunteers, with no evidence of immunosuppression or leukopenia. The pharmacokinetics of mizoribine were revealed to be very suitable for its intended use, with rapid and extensive oral availability, peak concentrations reached within 2–3 h, a short terminal elimination half-life in healthy volunteers of approximately 3 h and extensive renal excretion of >65% of the administered dose. The pharmacokinetics were also shown to be both independent of dose following single doses up to 12 mg kg^{-1}, and relatively linear with time between the first and last dose of multiple-dosing regimens up to 12 mg kg^{-1} day^{-1}. The mean trough concentration from the 12 mg kg^{-1} day^{-1} dosing range was above the minimum effective concentration of 0.5 μg ml^{-1} associated with sufficient inhibitory effect on organ rejection in the acute phase following renal transplantation once renal function returns to normal, while remaining well below the 3.0 μg ml^{-1} value associated with emergence of toxicities. Consequently, based on the results from the presented studies, it is being recommended that the starting dose for mizoribine in the up to 3 months acute phase following transplantation should be in the 6–12 mg kg^{-1} day^{-1} range and that the dose should be reduced only if the function of the transplanted kidney is impaired.

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