protein expression level was examined under fluorescence microscope. Approximately 48 h after transfection, cells were scraped from the dish in the presence of 1 mL cold phosphate-buffered saline. Cellular extracts were then prepared in 1× CHAPS lysis buffer as suggested by the manufacturer (Chemicon International, CA, USA). Telomerase activity of the cellular extract from 2×10^4 cells was assayed using the TRAPeze Telomerase Detection Kit following the manufacturer's directions (Chemicon International, CA, USA), except that PCR was performed as follows: 95 °C for 2 min; 25 cycles of 94 °C for 10 s, 50 °C for 30 s, 72 °C for 30 s; and 72 °C for 5 min. Products were analyzed on a 12% native polyacrylamide gel and examined by phosphor imaging (Molecular Dynamics, GE Healthcare Bio-Sciences Corp., NJ, USA).

Northern blotting analysis

Wild-type or mutant pcDNA3-hTERC vector (2 μg) was transfected into VA13+hTERT cells (at approximately 70% confluency) in 6-well polystyrene dishes using SuperFect transfection reagent. Approximately 48 h after transfection, Trizol reagent was used to extract total cellular RNA as suggested by the manufacturer (Invitrogen, CA, USA). Northern blot analysis was performed essentially as described [26].

Immunoprecipitation–Northern blotting analysis

FLAG-tagged hTERT protein was expressed in vitro from the pCR3-FLAG-hTERT vector using the TnT quick-coupled transcription-translation system (Promega) in the presence of 200 ng of in vitro-transcribed, gel-purified CR4–CR5 fragment of hTERC RNA spanning nucleotides 239 to 332 at 37 °C for 2 h. The resulting telomerase complexes were affinity-enriched on anti-FLAG agarose beads (Sigma, St. Louis, MO). To detect hTERT-bound telomerase RNAs, Northern blotting was performed on the enriched telomerase preparations as described above.

Results

Telomerase mutational analysis

We selected 100 BMFS patients who were diagnosed with either AA, mainly those who showed either only partial or no response to immunosuppressive therapy, or with MDS RA (Table 1). Genomic DNA from peripheral blood cells or marrow stem cells was extracted in order to amplify the *hTERT* and *hTERC* genes for sequencing. Even though we did not find any pathogenic mutations in the *hTERT* gene, we identified eleven polymorphic sequence changes (Table 2). These sequence polymorphisms were identified in both intronic and exonic regions of the gene and did not result in amino acid substitutions in the corresponding protein. Two of the polymorphic sequence changes (IVS6-93 G/A and codon837 CTC/CTG) have not been reported previously.

Table 2
Polymorphisms of *hTERT*

Exon	Substitution	No (%)
2	Codon305 GCA/GCG(Ala/Ala)	25
3	IVS3+130C/T	24
4	IVS3-24C/T	14
5	Codon699 GCC/GCT(Ala/Ala)	3
7	IVS6-93G/A	2
	Codon837 CTC/CTG(Leu/Leu)	1
9	Codon840 CTG/CTA(Leu/Leu)	3
	IVS9+11C/T	3
13	IVS13+45C/T	5
14	Codon1013 CAC/CAT(His/His)	13
15	IVSE14-94C/T	2

Table 3
Polymorphisms and mutation of *hTERC*

	AA, MDS ($n=100$)	Control ($n=120$)
Polymorphisms		
n-771A/G	11 (11.0%)	18 (15.0%)
n-714C insertion	12 (12.0%)	20 (16.7%)
Mutation		
n323C/T	1 (1.0%)	0 (0%)

In regard to the *hTERC* gene, we identified two novel heterozygous sequence polymorphisms in its promoter (n-771A/G and n-714 C insertion) at about a similar frequency in both patients and healthy controls (Table 3 and Fig. 1). On the contrary, we identified a novel heterozygous germ-line mutation in the *hTERC* gene (C322T) in an MDS patient only (Table 3 and Fig. 1). This patient was a 72-year-old man, who was clinically diagnosed with MDS RA. His bone marrow was slightly hypocellular but showed no sign of dysplasia or chromosomal abnormality. He showed a good and sustained response to metenolone and did not have a family history for the disease. Since this is an archival case, primary specimen (blood) collected from this patient did not yield an adequate amount of genomic DNA for measuring telomere lengths. The patient has deceased from ischemic heart disease.

Functional analysis of the hTERC C323T mutation

The novel C323T mutation changes a cytosine to a uracil in the hTERC RNA and is located on one strand of the P5 stem of the predicted hTERC RNA secondary structure (Fig. 2A) [27]. As such, we hypothesize that it may disrupt the basepairing interaction of this stem structure, which may lead to defective telomerase enzymatic function. In order to test this hypothesis, we introduced this hTERC natural variant C323T [or C323T(rt)] into a mammalian expression plasmid encoding the full-length (451-base) hTERC sequence. Because this specific nucleotide change might disrupt the basepairing interaction of the predicted P5 stem structure (Fig. 2A), we also created additional mutants designed to test the importance of this basepair. We designed a mutant denoted as C323T(lt) in which a guanine nucleotide located at position 246, on the opposite strand from the natural mutation, was mutated to an adenine (Fig. 2A). We also created a compensatory mutant [denoted as C323T(comp)] in which the natural C323T(rt) base mutation was accompanied by a complementary mutation on the opposite strand [C323T(lt)],

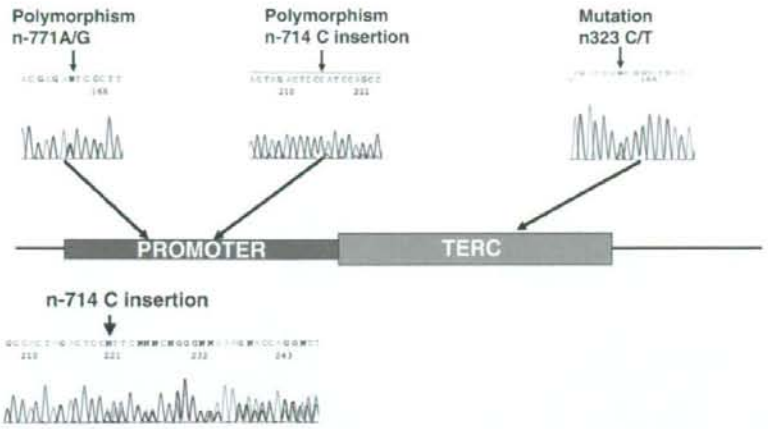

Fig. 1. Schematic depiction of the *hTERC* gene with naturally occurring sequence variations that occur in its promoter and coding sequence. Electrophereograms showing the heterozygous nature of these sequence variations are also shown. In the case of the n-714 C insertion, hTERC PCR product was subcloned into pCR2.1-TOPO expression vector before sequencing. Sequence analysis of PCR product of this sequence variant showing the heterozygous nature of the mutation is also shown in the bottom.

which would theoretically restore the predicted intramolecular basepairing interactions of the stem structure.

Biological activities of these hTERC variants were assessed by transient transfection of each vector into the VA13+hTERT cell line, a human lung-derived line that lacks endogenous hTERC but has been engineered to express stably the hTERT protein. These cells cannot ordinarily produce functional telomerase, but can assemble the active enzymatic complex when transfected transiently with a vector that expresses a functional hTERC copy. We prepared extracts of the cells approximately 48 h after transfection with the various hTERC constructs and estimated the steady-state levels of exogenous hTERC expression by Northern blot analysis. We then tested the extracts for telomerase enzymatic activity by measuring their ability to add telomeric DNA repeats onto a synthetic DNA primer in vitro, using a semi-quantitative, PCR-based telomere repeat amplification protocol (TRAP).

As compared to cell lysates that carry either the wild-type hTERC RNA or the one with a known inconsequential G58A polymorphic sequence change [16,19] (Fig. 2B, lanes 1–3 and 13–15), we found that, in each of the cases, the mutations located within the P5 stem drastically reduced telomerase enzymatic activity (lanes 4–12). More specifically, mutations located on the individual strands of the P5 stem [i.e., either the natural C323T(rt) mutation or the C323T(lt) mutation alone] abolished telomerase activity to about the same degree (Fig. 2B, lanes 4–9). Surprisingly, we found that the compensatory mutation C323T(comp) did not restore telomerase activity (lanes 10–12). Rather, this version of the RNA seemed to further reduce telomerase function in cells to an almost undetectable level. These results were not attributable to differences in hTERC RNA synthesis, processing or stability as Northern blotting verified that each construct produced comparable steady-state levels of hTERC expression in the transfected cells (Fig. 2C). As the disease-associated mutation is located within the highly conserved CR4–5 domain of hTERC that has been implicated as one of the hTERT-interacting sites, we asked whether our RNA mutants can also affect hTERT binding activity. Telomerase complexes were reconstituted in vitro using rabbit reticulocyte lysates to express a FLAG-tagged version of the hTERT protein in the presence of a synthetic hTERC RNA spanning nucleotides 239 to 332 of the CR4–5 domain. Anti-FLAG antibody was used to immunoprecipitate telomerase RNP complexes, which was used to probe for the CR4–5 RNA fragment. As shown in Fig. 2D, both the C323T(rt) and the C323T(comp) RNAs showed substantially impaired binding to hTERT protein. Taken together, these findings indicate that the disease-associated hTERC variant and its derivatives are functionally defective and that their defects may result from altering the conserved secondary structure and/or primary sequence of the RNA that abolishes its ability to interact with the hTERT catalytic protein component of telomerase.

hTERC C323T natural mutation functions as haploinsufficiency to modulate telomerase function

As the natural hTERC C323T variant was identified in an individual who is a heterozygous carrier for the gene, the altered allele might modulate normal telomerase function through either a haploinsufficiency or dominant negative fashion. In order to address this, we performed TRAP assays on cell lysates prepared from VA13+hTERT cells that had been co-transfected with plasmids to express the wild-type hTERC sequence and either the disease-associated hTERC C323T variant or the polymorphic G58A variant. As shown in Fig. 2B, little to no effects were observed between cells that carried only the wild-type hTERC vector and those that carried both the wild-type and the individual mutated hTERC copy (lanes 16–21), consistent with the idea that the natural variant functions in a haploinsufficiency manner to modulate wild-type telomerase function [5,19].

Discussion

This study shows that a novel variant telomerase RNA allele found in a Japanese patient with MDS is unable to support a normal level of telomerase enzymatic activity. This is consistent with the hypothesis that defects in telomerase function and telomere maintenance contribute to the pathogenesis of BMFS. This patient has deceased from ischemic heart disease. It has been documented that short telomeres may contribute to the pathophysiology of atherosclerosis and to the development of ischemic heart disease [28–30]. Some studies have implicated short telomere lengths to atherogenesis [31] and indicated that telomere lengths can serve as an effective marker for biological aging at a cellular level, such as aging cells of the vascular tissues [28–30].

The novel hTERC mutation reported in the current study is located on one side of the predicted P5 stem of the hTERC RNA secondary structure (Fig. 2A) that when altered drastically

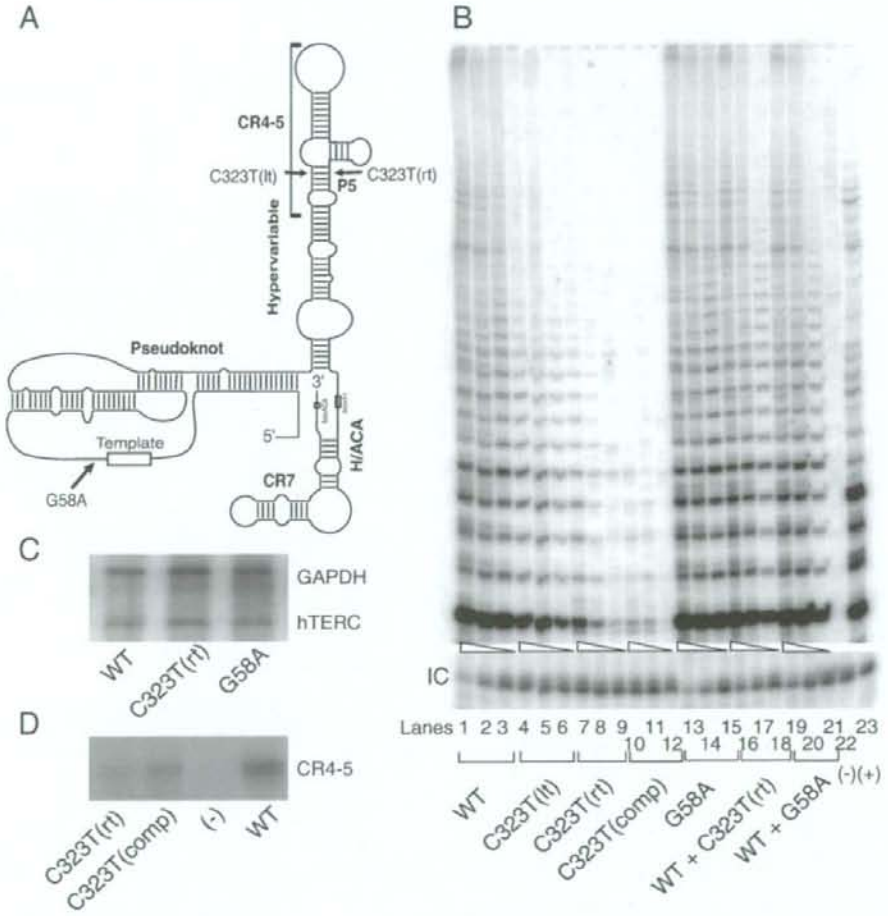

Fig. 2. (A) Schematic depiction of the predicted secondary structure of hTERC as proposed by Chen et al. [27]. The 8-base template sequence (rectangle) and other structural features are indicated, including the pseudoknot, CR4–CR5, box H/ACA and CR7 domains, as well as the hypervariable paired region. The inconsequential G58A polymorphism, disease-associated C323T mutation [aka C323T(rt)] and C323T(lt) engineered variant are shown. (B) Telomerase enzymatic activities as determined in VA13+hTERT cells for the naturally occurring hTERC mutation and its derivatives. A representative TRAP gel showing the relative telomerase enzymatic activities obtained from the substitution mutations and compensatory mutation. The compensatory mutation [C323T(comp)] was created in order to restore the P5 stem structure. Serial fivefold dilutions of the transfected cell lysates (indicated by triangles) were assayed for each sample to ensure linearity of the assay. Lane 22 shows a negative control composed of wild-type cell lysate denatured at 95 °C for 5 min prior to assay. Lane 23 shows PCR products amplified from the non-hTERC control TSR8 DNA template supplied in the TRAP kit. "IC" indicates PCR products amplified from an unrelated DNA template, which is included as an internal control for PCR amplification efficiency in each reaction. (C) Northern blot analysis of selected naturally occurring hTERC sequence variants and wild-type sequence expressed in transfected VA13+hTERT cells. Cellular GAPDH mRNA was assayed simultaneously. (D) Northern blot analysis of affinity-enriched telomerase complexes assembled using RNA fragments representing the CR4–5 domain of hTERC (Fig. 2A) with either the wild-type or mutated sequences and in vitro expressed hTERT catalytic protein. The negative control (lane 3) was a reaction that lacks the hTERT-expressing construct.

reduces telomerase enzymatic activity (Fig. 2B). More importantly, a combined substitution of both sides of this stem almost completely abolished telomerase function. It is noteworthy that the C323T variant allele described here is one of three known disease-associated alleles identified so far in this region (i.e., G322A, C323T and G305A), and each seems to effectively abolish telomerase function [20]. Therefore, it is possible that this region may serve as one of the hotspots for the natural process of mutagenesis that can result in defective telomerase function and telomere shortening effect observed in patients with hematologic disorders.

Our study indicates that this P5 region of the hTERC structure contributes to optimal telomerase function, and this intricately basepaired structure and/or its primary sequence serve as a critical feature for its biological activity. Indeed, it is striking that a high proportion of the seemingly minor point mutations examined in our earlier study, which aimed at comprehensively analyzing the structure and function of the entire hTERC molecule, severely compromised telomerase function by perturbing RNA structural formation [32]. However, in those cases, we found that compensatory mutations could fully restore telomerase activity, highlighting the importance of the normal basepairing pattern of the RNA. In the current study, we describe for the first time a unique region (P5) of the telomerase RNA molecule that seems to be highly sensitive to sequence alteration. As this region is part of the conserved CR4–5 region (Fig. 2A) that has been shown to serve as one of the sites for the catalytic hTERT protein to assemble onto the RNA [20,33,34], we show here that indeed both the disease-associated mutant C323T(rt) and the compensatory C323T(comp) mutant substantially impair hTERT binding. Our data, therefore, support the idea that even a minute sequence change in this region can perturb this critical telomerase ribonucleoprotein interaction and hence abrogate its enzymatic activity.

Together with earlier reports [4,17,20,26,32], our data presented here are consistent with the idea that defects in telomerase function and telomere maintenance contribute to the pathogenesis of BMFS in a subset of these patients. As we found only one patient with a potential pathogenic mutation in the *hTERC* gene and none with pathogenic *hTERT* mutations among a cohort of 220 Japanese men and women, our study revealed that natural sequence variations in telomerase gene components rarely occurred in the Japanese population. This is consistent with a recent finding of no mutations in the *hTERC* gene in 35 Japanese MDS patients and 134 healthy volunteers [23]. A separate group examined 96 Japanese children with acquired AA and 76 healthy controls and similarly found no mutations in the *hTERC* gene [20]. However, this study revealed two nonsynonymous mutations in the *hTERT* gene among several inconsequential polymorphic sequence changes in this gene. Collectively, these studies showed that mutational frequencies of telomere-synthesizing genes among Japanese BMFS patients were lower than what had been reported for other ethnic groups [5,7,16,17] and that this genetic difference could not explain the higher incidence of the disease in Asian populations.

Acknowledgments

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ORIGINAL ARTICLE

A safety, pharmacokinetic and pharmacodynamic investigation of deferasirox (Exjade®, ICL670) in patients with transfusion-dependent anemias and iron-overload: a Phase I study in Japan

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Abstract The pharmacokinetics (PK) and pharmacodynamics (PD) of the once-daily, oral ironchelating agent, deferasirox (Exjade®, ICL670), have been evaluated further in a Phase I, openlabel, multicenter, dose-escalation study in Japanese patients with myelodysplastic syndromes, aplastic anemia, and other anemias. Deferasirox was initially administered as a single dose of 5 ($n = 6$), 10 ($n = 7$), 20 ($n = 6$) or 30 ($n = 7$) mg/(kg day) and then after 7 days seven daily doses were administered. Linear PK (C_{max} and AUC) were observed at all doses after a single dose and at steady state, and dose-dependent iron excretion was observed. Pharmacokinetic/pharmacodynamic parameters were similar to those reported in a Caucasian β-thalassemia cohort. Following the single- and multiple-dose phases, 21 of 26 patients progressed to a 3-year extension phase of the study, where dose reductions and increases [5–30 mg/(kg day)] were allowed following safety and efficacy assessments. In the interim, 1-year data show that deferasirox was well tolerated, with generally infrequent and mild adverse events. Reductions in serum ferritin levels were observed and a negative iron balance achieved at doses of 20–30 mg/(kg day). These data suggest that deferasirox has a stable and predictable PK/PD profile, irrespective of underlying disease or race, and a predictable and manageable safety profile suitable for chronic administration.

Keywords Iron chelation · Deferasirox · Iron overload · Myelodysplastic syndrome

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1 Introduction

Blood transfusions provide key supportive therapy for patients with chronic anemias, relieving the disease symptoms, improving quality of life, and extending survival [1–3]. However, the senescence of transfused red blood cells leads to the release of excess iron for which the body has no active mechanism of removal. Once levels of transferrin, an iron-binding protein, are saturated, free iron begins to circulate and is taken up and stored in the

parenchymal cells of the liver, heart, endocrine organs, brain, and joints [4, 5]. The accumulation of toxic iron leads to the generation of active oxygen species, resulting in DNA damage, lipid peroxidation, apoptosis and, therefore, ongoing tissue damage. If iron levels remain uncontrolled, progressive organ dysfunction will result in death, principally from cardiac and liver failure. Iron overload can be effectively managed with iron chelation therapy, which can prevent the consequences of iron toxicity and improve patients' long-term outcomes [6–9].

In Japan, the myelodysplastic syndromes (MDS) and aplastic anemia (AA) are the most common anemias requiring regular blood transfusion therapy. Despite widespread recognition of the risks associated with iron overload, and the subsequent need to manage iron levels, treatment options are, at present, limited to deferoxamine (Desferal®, DFO), an iron chelator with a high molecular weight that requires parenteral administration. In contrast to most countries around the world where DFO is administered via slow subcutaneous infusion utilizing a portable pump because of its short half-life, DFO is approved in Japan only for intravenous and intramuscular administration. In contrast to the recommended regimen of slow subcutaneous infusion 5–7 nights/week, it is therefore a common practice for DFO to be administered only once every 2 weeks in a hospital setting. As studies have demonstrated that chelation coverage is limited to periods of drug exposure, infrequent infusions will allow raised levels of non-transferrin bound iron to reoccur, exposing patients to toxic iron levels [10, 11]. A recent survey on Japanese patients with MDS, AA, and other anemias, highlighted that less than half had received iron chelation therapy, and of those who were treated, less than 9% received continuous/daily DFO therapy [12]. A high mortality rate was noted in this population, primarily from cardiac and liver failure, conditions commonly associated with iron overload [5, 13]. Daily or continuous iron chelation therapy was seen to effectively reduce iron burden and improve organ function [12].

Once-daily oral therapy with deferasirox (Exjade®, ICL670) has the potential to overcome the limitations of DFO treatment, providing convenient therapy and 24-h chelation coverage [14]. Registration studies conducted on non-Japanese adult and pediatric patients have demonstrated a similar efficacy to DFO at comparable doses, and dose-dependent efficacy in reducing body iron burden across a wide range of transfusion-dependent anemias [15–18]. Deferasirox has since been approved in more than 85 countries worldwide. Here, we present the findings of a Phase I clinical trial on Japanese patients with transfusion-dependent anemias treated with deferasirox in an initial pharmacokinetic (PK)/pharmacodynamic (PD) study, and interim analyses of data from the subsequent extension phase. The PK/PD parameters were compared with those previously reported in a cohort of iron-overloaded Caucasian patients with β-thalassemia [19].

2 Methods

2.1 Study objectives

The primary objective was to evaluate the tolerability and safety of deferasirox in Japanese patients with transfusional iron overload. Secondary objectives were to evaluate the PK and PD of deferasirox, including iron excretion. A comparison of the PK/PD data with those of a previously published Phase I trial (study 0104) conducted on non-Japanese β-thalassemia patients was also performed [19].

2.2 Patients

Eligible patients were ≥20 years of age, with transfusion-dependent MDS, AA or other anemias (pure red cell aplasia: PRCA, myelofibrosis: MF), having received a lifetime history of ≥35 U of packed red blood cells (RBCs). In Japan, 1 U of RBCs contains 200 mL of whole blood, and provides approximately 100 mg of iron. Serum ferritin values ≥1,000 μg/L as confirmed by at least two evaluations, during the 4 weeks prior to enrollment and an ECOG performance value of 0–2 were required. As a result, the patient with chronic inflammation, for example adult Still disease or hemophagocytic syndrome, did not enroll in this study. Patients receiving DFO therapy during the 4 weeks prior to the start of deferasirox treatment were excluded. Other parameters that excluded patients from the study at screening included alanine aminotransferase (ALT) levels >250 U/L, serum creatinine levels above the upper limit of normal (ULN), a urinary protein/creatinine aplastic anemia ratio >0.5 mg/mg, serological evidence of chronic hepatitis B virus infection, clinical evidence of active hepatitis C virus infection, uncontrolled gastrointestinal problems (diarrhea, constipation, or bleeding), and cataract or a previous history of clinically relevant ocular dysfunction related to iron chelation. All patients provided written informed consent.

The study by Nisbet-Brown et al. [19] (Study 0104), which was used for comparison, enrolled Caucasian patients (male and female, aged ≥16 years) with β-thalassemia and transfusional iron overload.

2.3 Study design and dosing

This Phase I, collaborative, openlabel, non-blind, dose-escalation study was conducted in nine centers in Japan. The study was conducted in three phases, a single-dose phase (1-day treatment in each dose cohort), a multiple-dose

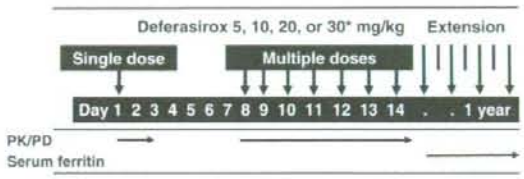

Fig. 1 Study design indicating dosing and PK/PD assessments. *Asterisk* denotes cohort commenced treatment on 20 mg/(kg day) in the extension

phase (7-day treatment in each dose cohort), and an extension phase (1-year data reported; Fig. 1). Single dosing was initiated with a cohort treated at 5 mg/kg. Efficacy and Safety Committee review subsequently approved enrollment of the next dosing cohort (10, 20, and 30 mg/kg) and progression to the multiple-dose phase where patients received daily doses of deferasirox 30 min before breakfast. Deferasirox dosing was based on previous studies in Caucasian populations [19, 20]. Patients were hospitalized during the single- and multiple-dose periods and given a standard low iron diet from day 1 to day 14. In study 0104, deferasirox was administered at 10, 20, and 40 mg/kg once daily for 12 days [19].

Following completion of the single- and multiple-dose phases of the study, treatment could continue into the extension phase for approximately 3 years at the patient's request in the ethical point of view for patient. Following Efficacy and Safety Committee review, a recommendation to adjust deferasirox dose in relation to transfusion requirement was made. As a result, patients receiving deferasirox 5, 10 or 20 mg/(kg day) continued into the extension phase at the same doses, but those who had originally received 30 mg/(kg day) began the extension phase on 20 mg/(kg day) to avoid potential over chelation. Dose adjustment was subsequently allowed to 30 mg/(kg day) in the extension phase following a trend of increasing serum ferritin levels or increased frequency of blood transfusion. Dose was decreased down to 5 mg/(kg day) in cases of increasing serum creatinine levels, adverse events (AEs) or decreases in serum ferritin level. Dose reduction or interruptions were implemented for skin rash, increase in serum creatinine, and increase in urinary protein/creatinine ratio. If serum ferritin fell to $\leq$500 μg/L on two consecutive study visits, treatment was interrupted until serum ferritin was >1,000 μg/L.

2.4 Safety assessments

Safety evaluations were based on reports of AEs and serious AEs, plus assessment of hematology, blood chemistry, urinalysis, vital signs, physical examinations, electrocardiograms, and ocular and audiometry examinations.

2.5 Pharmacokinetic/pharmacodynamic evaluation

Pharmacokinetic/pharmacodynamic assessments were made during the single- and multiple-dose phases. Pharmacokinetic calculations were based on free deferasirox and the iron-complex of deferasirox (Fe-[ICL670]$_2$) by means of a non-compartmental analysis to determine t_{max}, C_{max}, AUC_{0-24}, and $t_{1/2}$. Total iron excretion was calculated as the sum of the urinary and fecal iron excretion. Iron excretion induced by deferasirox was calculated as the difference between an average daily iron excretion during the treatment period (days 11, 12, 13, and 14) and an average daily iron excretion during the cessation period (days 5, 6, and 7) as the baseline value.

2.6 Markers of iron stores

During the extension phase, safety, serum ferritin, and blood biochemical tests [including albumin, alkaline phosphatase, total bilirubin, blood urea nitrogen (BUN), cholesterol, creatinine, γ-GTP, glucose, LDH, total protein, ALT, AST, triglycerides, uric acid, CRP, sodium, potassium, chloride, calcium, inorganic phosphorus, and magnesium] were evaluated monthly. Exploratory examination of changes from baseline in serum ferritin levels was conducted.

3 Results

3.1 Patient demographics

Twenty-six patients with MDS, AA or other anemias were enrolled in the single- and multiple-dose phases, all of whom completed the core phase of the study (Table 1). Twenty-one patients continued to the extension phase. Study 0104, which was used to compare the PK/PD results from the current study, enrolled nine male and nine female Caucasian patients with β-thalassemia with a median age of 26.4 years (range 18–39) [19], lower than the median age of 69.5 years (range 26–93) of the Japanese patients in the current study.

3.2 Dosing and exposure to deferasirox

All 26 patients completed the single- and multiple-dose phases. Of these 21 patients who wished to continue deferasirox treatment as extension phase, 14 (66.7%) remain on study treatment for 1 year and are continuing in the study. Those who withdrew did so primarily due to AEs or improvements in serum ferritin value that meant chelation therapy was no longer required (Table 2).

Adjustments to the starting dose were made due to insufficient efficacy of the initial dose as assessed by serum ferritin (increase in dose) and AEs (decrease in dose;

Table 1 Patient characteristics

	Deferasirox dose [mg/(kg day)]				
Core phase	5 ($n = 6$)	10 ($n = 7$)	20 ($n = 6$)	30 ($n = 7$)	Total ($n = 26$)
Median age, years (range)	71.5 (28–78)	68.0 (34–93)	66.0 (26–74)	75.0 (46–87)	69.5 (26–93)
Male:female	1:5	3:4	1:5	3:4	8:18
Underlying anemia, *n* (%)					
MDS	3 (50)	5 (71.4)	4 (66.7)	4 (57.1)	16 (61.5)
AA	3 (50)	1 (14.3)	1 (16.7)	1 (14.3)	6 (23.1)
Other anemias[a]	0	1 (14.3)	1 (16.7)	2 (28.6)	4 (15.4)

Extension phase	5 ($n = 5$)	10 ($n = 5$)	20 ($n = 11$)	Total ($n = 21$)
Median age, years (range)	70.0 (28–77)	68.0 (34–78)	68.0 (26–76)	68.0 (26–78)
Male:female	1:4	2:3	3:8	6:15
Underlying anemia, *n* (%)				
MDS	2 (40.0)	3 (60.0)	7 (63.6)	12 (57.1)
AA	3 (60.0)	1 (20.0)	2 (18.2)	6 (28.6)
Other anemias[b]	0	1 (20.0)	2 (18.2)	3 (14.3)
Blood intake during the extension phase, median units/month[c] (range)	4.4 (0–9.6)	4.3 (0–6.1)	3.9 (3.0–13.2)	4.4 (0–15.7)

MDS, myelodysplastic syndromes; *AA*, aplastic anemia

[a] Other anemias: pure red cell aplasia ($n = 3$), myelofibrosis ($n = 1$)

[b] Other anemias: pure red cell aplasia ($n = 2$), myelofibrosis ($n = 1$)

[c] 1 unit = 200 mL

Table 2). As a result of the criteria of dose reduction and interruption shown in Sect. 2.3, dosing was reduced and interrupted in five (23.8%) and eight (38.1%) patients, respectively, because of adverse events (AEs). Seven patients (33.3%) stopped treatment during the extension phase: four (19.0%) because of AEs; two (9.5%) because of improvements in their serum ferritin values; and one (4.8%) because of insufficient nutrition due to family circumstances. In study 0104, five, six, and seven patients were dosed with deferasirox 10, 20, and 40 mg/(kg day), respectively [19].

3.3 Safety and tolerability

Deferasirox was generally well tolerated, and reported AEs were infrequent and mild in severity. No deaths were reported during the study. In the single- and multiple-dose phase, the most common AEs with a reported relationship to deferasirox were diarrhea [7.7%; $n = 2$ each after single and multiple doses of 30 mg/(kg day)], nausea [7.7%; $n = 1$ each after multiple doses at 10 and 30 mg/(kg day)], and non-progressive increases in serum creatinine [7.7%;

Table 2 Duration of treatment, dose adjustments, and discontinuations during the extension phase

	Deferasirox dose [mg/(kg day)]			
	5 ($n = 5$)	10 ($n = 5$)	20 ($n = 11$)	Total ($n = 21$)
Median treatment duration, days (range)	361 (231–365)	365 (197–365)	365 (71–374)	365 (71–374)
Dose adjustments				
Increase, *n* (%)	3 (60.0)	3 (60.0)	1 (9.1)	7 (33.3)
Decrease due to AEs, *n* (%)	0	0	5 (45.5)	5 (23.8)
Interruption due to AEs, *n* (%)	2 (40.0)	2 (40.0)	4 (36.4)	8 (38.1)
Interruption due to symptom improvement, *n* (%)	1 (20.0)	0	2 (18.2)	3 (14.3)
Discontinuations				
Symptom improvement, *n* (%)	1 (20.0)	1 (20.0)	0	2 (9.5)
AEs, *n* (%)	1 (20.0)	1 (20.0)	2 (18.2)	4 (19.0)
Family circumstances[a], *n* (%)	1 (20.0)	0	0	1 (4.8)

AEs, adverse events

[a] Insufficient nutrition

$n = 1$ each after multiple doses at 5 and 30 mg/(kg day)]. One of the 26 patients (3.8%) had two serious AEs, pyrexia and duodenal ulcer, during the multiple-dose phase after doses of 30 mg/(kg day). A relationship with study drug could not be excluded as a cause of this event, although the patient had experienced occasional upper GI symptoms before starting the study.

During the extension phase, the most common AEs with a reported relationship to deferasirox were non-progressive increases in serum creatinine (>33% from baseline value or >ULN at two consecutive visits), increased urine β2-microglobulin, and increased blood alkaline phosphatase (Table 3). Increases in serum creatinine were seen most frequently in the 20 mg/kg dose group, 4–8 weeks after the start of treatment. In patients with increased serum creatinine, a dose reduction or interruption was required most frequently in patients in the higher (20 mg/kg) dose group whose amount of blood transfusion was low [<7 mL/(kg month)].

One of the 21 patients receiving deferasirox 20 mg/(kg day) had a transient increase in liver transaminase levels. Deferasirox treatment was interrupted for 1 week, which led to a reduction in transaminase levels. Deferasirox was restarted at 20 mg/(kg day), and no subsequent increase in transaminases was observed; treatment continued at the same dose.

There were two serious AEs with a suspected relationship to deferasirox administration, both in patients treated at 20 mg/(kg day): one of the 21 patients suffered pharyngeal ulceration and another had interstitial nephritis. Deferasirox therapy was stopped in both cases. The patient with pharyngeal ulceration had a pharyngeal tumorectomy and recovered 7 months after removal from the study, while in the case of interstitial nephritis, the patient's serum creatinine and BUN improved but she was removed from the study 10 weeks after the start of the treatment because her serum creatinine did not return to the initial value. Two other dose discontinuations were due to dementia and progressive multifocal leukoencephalopathy, and these were unrelated to study drug.

3.4 Pharmacokinetic and pharmacodynamic evaluation

3.4.1 Pharmacokinetic evaluation

After single and multiple doses (at steady state), dose-proportional C_{max} and AUC were observed (Table 4; Fig. 2). At steady-state, C_{max} and AUC were approximately 1–2-fold higher than following single-dose administration. The PK parameters (C_{max} and AUC) of deferasirox measured in these Japanese patients were

Table 3 Treatment-related adverse events occurring in ≥2 patients in the extension phase

	Initial deferasirox dose [mg/(kg day)]			
	5 ($n = 5$)	10 ($n = 5$)	20 ($n = 11$)	Total ($n = 21$)
Total adverse drug reactions, *n*	0	1 (20.0)	10 (90.9)	11 (52.4)
Increased serum creatinine[a], *n* (%)	0	0	6 (54.5)	6 (28.6)
Increased urine β2-microglobulin, *n* (%)	0	0	4 (36.4)	4 (19.0)
Increased serum alkaline phosphatase, *n* (%)	0	0	3 (27.3)	3 (14.3)

[a] Non-progressive increase >33% from baseline value or >ULN at two consecutive visits

Table 4 Pharmacokinetic parameters of deferasirox after the single- and multiple-dose phases

Dose (mg/kg)	t_{max} (h)	C_{max} (μmol/L)	AUC_{0-24} (μmol h/L)	$t_{1/2}$ (h)
Day 1 (single dose)				
5 ($n = 6$)	2.0 (0.9–3.0)	20.4 ± 6.1	190 ± 91	8.5 ± 3.4
10 ($n = 7$)	3.0 (1.0–4.0)	53.3 ± 18.7	535 ± 137	17.1 ± 4.7
20 ($n = 6$)	4.0 (1.0–10.0)	112 ± 29	1,270 ± 366	20.5 ± 4.9
30 ($n = 7$)	3.0 (2.0–4.0)	119 ± 40	1,450 ± 423	18.9 ± 9.8[a]
Day 14 (multiple dose)				
5 ($n = 6$)	1.5 (1.0–4.0)	27.4 ± 10.7	345 ± 236	17.5 ± 7.2
10 ($n = 7$)	3.0 (1.1–10.0)	67.3 ± 22.2	848 ± 442	20.5 ± 7.5
20 ($n = 6$)	3.4 (1.0–4.2)	119 ± 14	1,510 ± 193	21.4 ± 7.2
30 ($n = 7$)	3.9 (1.0–10.0)	224 ± 100	3,620 ± 2,760	19.5 ± 4.9

Mean ± SD except for t_{max} [median (min–max)]

[a] $n = 6$

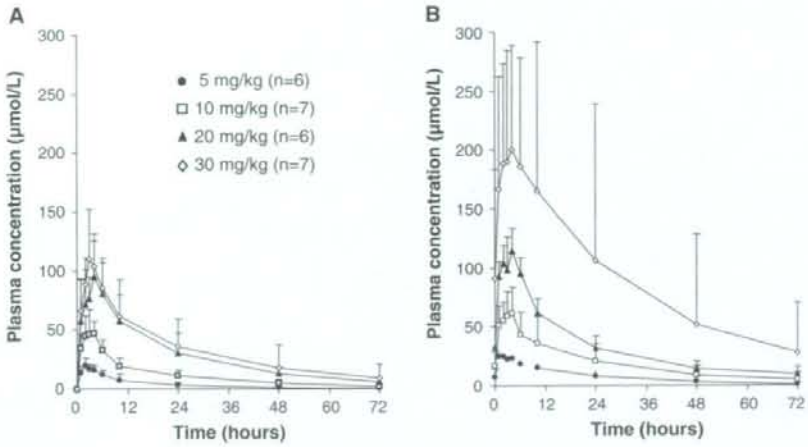

Fig. 2 Plasma concentration–time profiles of deferasirox after **a** single dosing (day 1) and **b** multiple dosing (day 14)

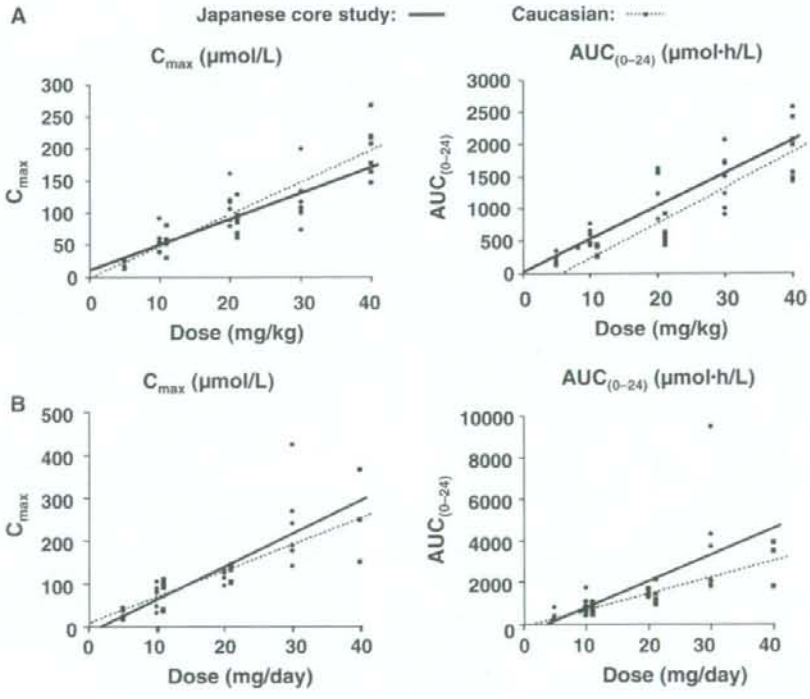

Fig. 3 pharmacokinetics–dose relationship in Japanese (data from current study) and Caucasian patients [19] (data from study 104) after **a** single dosing (day 1) and **b** multiple dosing (day 14)

similar to those measured in Caucasian patients in study 0104 (Fig. 3) [19].

3.4.2 Pharmacodynamic evaluation

One patient in the 5 mg/kg dosing group experienced fecal occult blood and was excluded from the PD data analysis. Dose-dependent iron excretion and a linear relationship between PK (AUC) and PD (iron excretion) were observed (Table 5), with iron excretion being similar to that measured in the Caucasian patients in study 0104 (range 0.12–0.45 mg iron/(kg day); Figs. 4, 5).

3.5 Changes in serum ferritin levels

Changes in levels of serum ferritin were assessed as a marker of iron stores. At the start of the extension phase, median serum ferritin levels were 4,500 μg/L (range

Table 5 Iron excretion

Dose (mg/kg)	Mean iron excretion rate ± SD [mg/(kg day)]		
	Fecal iron excretion	Urinary iron excretion	Total iron excretion
5 ($n = 6$)	0.07 ± 0.10	0.01 ± 0.00	0.07 ± 0.10
10 ($n = 7$)	0.12 ± 0.12	0.01 ± 0.00	0.13 ± 0.12
20 ($n = 6$)	0.33 ± 0.12	0.02 ± 0.00	0.34 ± 0.12
30 ($n = 7$)	0.58 ± 0.39	0.02 ± 0.01	0.61 ± 0.39

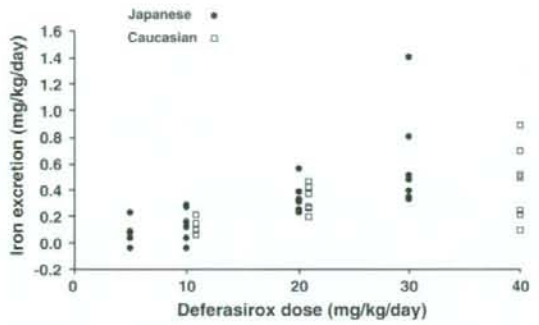

Fig. 4 Dose-related iron excretion in Japanese (data from current study) and Caucasian patients [19] (data from study 104)

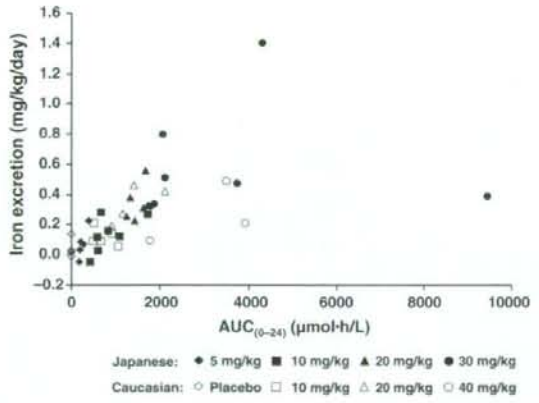

Fig. 5 Relationship between PK and PD in Japanese (data from current study) and Caucasian patients [19] (data from study 104)

1,400–17,000). After 1 year, all patients in the 20 mg/(kg day) group had a decrease in their serum ferritin levels, indicating a negative iron balance, as did four of five patients in the 10 mg/(kg day) group and two of five patients in the 5 mg/(kg day) group. However, these results include dose increases according to the dose adjustment protocol: in the 5 mg/(kg day) group, two patients were increased to 10 mg/(kg day) and one patient was increased

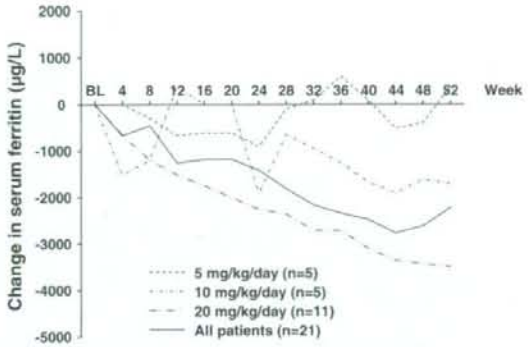

Fig. 6 Changes in serum ferritin following administration of deferasirox. Dose was increased according to the dose adjustment protocol: in the 5 mg/(kg day) group, two patients were increased to 10 mg/(kg day) and one patient was increased to 20 mg/(kg day); in the 10 mg/(kg day) group, three patients were increased to 20 mg/(kg day); and in the 20 mg/(kg day) group, one patient was increased to 30 mg/(kg day)

Table 6 The value of serum ferritin in baseline and after 1 year of deferasirox

Dose (mg/kg)	Mean value of serum ferritin ± SD (μg/L)			
	Baseline		After 1 year (52 weeks)	
5 ($n = 5$)	($n = 5$)	3,700 ± 2,203	($n = 3$)	4,267 ± 1,012
10 ($n = 5$)	($n = 5$)	7,040 ± 5,719	($n = 3$)	4,533 ± 1,747
20 ($n = 11$)	($n = 11$)	4,309 ± 1,865	($n = 8$)	1,243 ± 496
All ($n = 21$)	($n = 21$)	4,814 ± 3,307	($n = 14$)	2,596 ± 1,843

to 20 mg/(kg day); in the 10 mg/(kg day) group, three patients were increased to 20 mg/(kg day); and in the 20 mg/(kg day) group, one patient was increased to 30 mg/(kg day). The median serum ferritin level after 1 year of deferasirox treatment fell by 3,485 μg/L in the 20 mg/(kg day) group and by 1,700 μg/L in the 10 mg/(kg day) group; the median serum ferritin level rose by 400 μg/L in the 5 mg/(kg day) group (Fig. 6). The mean values of serum ferritin at each dose of deferasirox in baseline and after 1 year of deferasirox treatment is shown in Table 6.

4 Discussion

Deferasirox is a once-daily oral iron chelator that allows flexible dosing, adjustable to transfusional iron intake, and therapeutic goal (decrease or maintenance of total body iron). This study assessed the safety, PK, and PD of deferasirox in Japanese patients with chronic anemias and transfusion-dependent iron overload in order to compare with data obtained in Caucasian patients with transfusion-dependent β-thalassemia and secondary iron overload [19].

Deferasirox was generally well tolerated with a manageable safety profile after daily dosing for up to 1 year. The incidence and severity of AEs related to study treatment appeared to be dose dependent, although low patient numbers in each group limit a conclusion. The AE profile in Japanese patients was similar to that seen in previous studies of Caucasian patients with MDS, with no additional safety concerns [15]. Potential AEs in Japanese patients could, therefore, be expected to be anticipated and managed in a similar way to those in non-Japanese patients [21]. Mild increase of serum creatinine to >33% above baseline levels and interstitial nephritis has been observed in the patients treated at 20 mg/kg. After the initial increase, the creatinine levels have remained stable and transient increases in the urinary excretion of β2-microglobulin were observed in some patients. These findings may relate to pre-existing proximal renal tubular damage, which has been attributed to the toxic effects of iron deposits in the kidneys. Investigations are ongoing to explore the effects of deferasirox on the kidney. Meanwhile, serum creatinine should be monitored monthly in all patients.

Exposure to deferasirox was dose dependent with a linear PK/PD relationship, resulting in dose-dependent iron excretion. The PK/PD parameters in the Japanese patients were similar to those seen in the Nisbet-Brown et al. Caucasian β-thalassemia cohort (Study 0104), suggested that deferasirox has a stable and predictable PK/PD, regardless of underlying disease or race. Although efficacy was not evaluated in this study, assessment of serum ferritin levels as an indicator of iron stores indicates that the high iron load of this patient population was reduced after 1 year's deferasirox therapy, and a negative iron balance was achieved in the 20 mg/(kg day) dose group. In a previous study of deferasirox in non-Japanese patients with MDS, and a similar mean iron intake level to the Japanese patients in the current study, doses of 20 and 30 mg/(kg day) were also shown to maintain or reduce iron levels.

In conclusion, this study demonstrates that the PK/PD profile of deferasirox in iron-overloaded Japanese patients with transfusion-dependent anemias is consistent with that previously reported in Caucasian patients [19], with safety and tolerability profiles similar to previous deferasirox studies [21].

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Japanese epidemiological survey with consensus statement on Japanese guidelines for treatment of iron overload in bone marrow failure syndromes

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Abstract Many patients with bone marrow failure syndromes need frequent transfusions of red blood cells, and most of them eventually suffer from organ dysfunction induced by excessively accumulated iron. The only way to treat transfusion-induced iron overload is iron chelating therapy. However, most patients have not been treated effectively because daily/continuous administration of deferoxamine is difficult for outpatients. Recently, a novel oral iron chelator, deferasirox, has been developed, and introduction of the drug may help many patients benefit from iron chelation therapy. In this review, we will discuss the current status of iron overload in transfusion-dependent patients, and the development of Japanese guidelines for the treatment of iron overload in Japan, which were established by the National Research Group on Idiopathic Bone Marrow Failure Syndromes in Japan.

Keywords Bone marrow failure syndrome · Iron overload · Iron chelation · Guidelines

1 Introduction

Many patients with aplastic anemia (AA) or myelodysplastic syndromes (MDS) need frequent transfusions of red blood cells (RBCs). One unit (derived from 200 mL of whole blood) of RBC transfusion in Japan contains about 100 mg of iron. Because there is no physiological mechanism for iron excretion in humans, and daily iron excretion is no more than 1 mg in a healthy man, repeated RBC transfusions will soon result in iron overload. Excess iron is mainly deposited in the liver, heart and pancreas, and causes organ dysfunction [1, 2].

As phlebotomy is not an option because of the underlying bone marrow failure, the only way to treat iron overload is by iron chelation therapy. However, difficulty in optimal administration of deferoxamine (DFO, Desferal®) in Japan has hampered effective chelation, and currently most patients are not treated effectively [3].

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Recently, a novel oral iron chelator, deferasirox (Exjade®), has been introduced in more than 60 countries, including Japan. The introduction of deferasirox may improve compliance with iron chelation therapy [4]. Under these circumstances, the National Research Group on Idiopathic Bone Marrow Failure Syndromes in Japan drew up Japanese guidelines for the treatment of transfusion-induced iron overload. Herein, we describe the current status of iron overload in transfusion-dependent patients in Japan, and development of the proposed guidelines for the treatment of transfusion-induced iron overload.

2 Current status of transfusion-induced iron overload in Japan

In 2005, the first nationwide survey on iron overload in transfusion-dependent patients in Japan was carried out [3]. This retrospective survey investigated the outcomes of iron overload-related morbidity and mortality from August 2001 to December 2005. A questionnaire was sent to hematology departments in hospitals all over Japan, and 43 hospitals responded by returning data on 292 patients.

Demographic data showed that MDS and AA accounted for about 80% of the underlying diseases: MDS, 52.1%; AA, 30.8%; pure red cell aplasia (PRCA), 5.1%; and myelofibrosis (MF), 4.5%. Serum ferritin levels were significantly correlated with the lifetime total number of RBC transfusion units received. Figure 1 shows the relationship between the number of RBC units and mean ferritin level, indicating the percentage of patients with an abnormal ferritin level ($\geq$1,000 ng/mL) for any total number of RBC units received as analyzed by a logistics model. The goodness-of-fit of this model between theoretical and actual values was assessed by Pearson chi-squared test, and the estimated number of RBC units required to raise ferritin to $\geq$1,000 ng/mL in 50 and 75% of patients was calculated as 21.5 and 43.4 units, respectively.

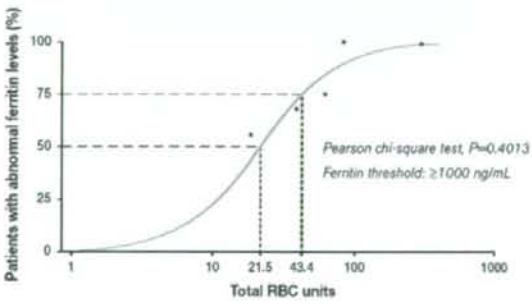

Fig. 1 Relationship between serum ferritin and total number of red blood cell units. [3] Modified with permission from Takatoku et al. *Eur J Haematol.* 2007;78:487–494. ©2007 Blackwell Publishing

Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) abnormalities were significantly correlated with transfusion frequency and increased ferritin levels; there was a significantly ($P < 0.0001$) higher prevalence of SGOT and SGPT abnormality in patients with high serum ferritin than in those whose serum ferritin was <1,000 ng/mL (Fig. 2). Moreover, among patients in whom cardiac function was evaluated, abnormalities were found in 21.9%, and cardiac abnormality was weakly correlated with serum ferritin levels. These data indicate that ferritin levels can be a useful predictor of hepatic and cardiac dysfunction. Fasting blood sugar (FBS) abnormality was also correlated with transfusion frequency.

In the survey, 75 deaths were reported, most of which were caused by infection and leukemia. However, cardiac and hepatic failure was noted in 24% and 6.7% of cases, respectively. Patients who died from cardiac or hepatic failure had received more transfusions than those who died from other causes, and among 38 patients in whom serum ferritin levels were available, 37 patients died with serum ferritin levels $\geq$1,000 ng/mL; the majority of patients (24 patients) had serum ferritin levels >5,000 ng/mL. These data indicate that multiple transfusion therapy is associated with a high risk of fatal complications caused by iron overload. Recently, similar analyses have been reported describing that transfusion-dependent MDS patients show significantly shorter survival than those who do not require transfusions and that transfusion-induced iron overload significantly affects survival [5].

3 Iron chelation therapy

As phlebotomy is not an option because of underlying bone marrow failure, the only way to treat iron overload is with iron chelation therapy. Until recently, the only available iron chelating agent in Japan was DFO. Because of the limited absorption from the gastrointestinal tract and short biological half-life of the agent, the drug must be administered by parenteral injections at least 5–7 times a week, or continuously for optimal effectiveness [6]. In the survey, 43.2% of patients received DFO, but only 8.6% received DFO daily or continuously; most of the patients were administered the drug intermittently (average once per 1.9 weeks) or concurrently with transfusion [3]. While improvements in serum ferritin, SGOT, SGPT and FBS were noted in the patients who received DFO daily or continuously, these data did not improve, and rather worsened, in those without optimal administration (Table 1). This indicates that appropriate administration of the chelating agent is needed for sufficient therapeutic results.

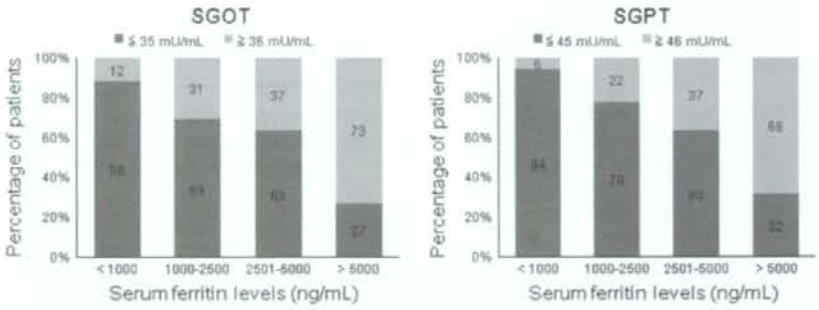

Fig. 2 Relationship between serum transaminase abnormality and serum ferritin levels. [3] Modified with permission from Takatoku et al. *Eur J Haematol.* 2007;78:487–494. ©2007 Blackwell Publishing

Table 1 Average changes in laboratory values during the period of transfusion dependence in patients receiving deferoxamine treatment

Parameter	Intermittent (once/1.9 week)	Concurrent with transfusion	Daily/continuous
Serum ferritin[a,b] (ng/mL)	+2222.8 ($n = 36$)	+2204.8 ($n = 19$)	−1135.2 ($n = 9$)
SGOT[a,c] (mU/mL)	+28.0 ($n = 53$)	+40.0 ($n = 30$)	−9.2 ($n = 10$)
SGPT (mU/mL)	+28.6 ($n = 53$)	+10.3 ($n = 30$)	−28.8 ($n = 10$)
FBS (mg/dL)	+31.2 ($n = 31$)	+8.2 ($n = 12$)	−4.8 ($n = 5$)

[3] Modified with permission from Takatoku et al. *Eur J Haematol.* 2007;78:487–494. ©2007 Blackwell Publishing

[a] Intermittent versus continuous, $P < 0.05$

[b] Continuous versus concurrent, $P < 0.01$

[c] Continuous versus concurrent, $P < 0.05$

Moreover, it has also been reported that iron chelation not only reduced iron burden and improved organ dysfunction, but also ameliorated the hemoglobin levels of iron-overloaded patients [7, 8]. Although the biological mechanism of the hematopoietic recovery remains to be elucidated, this fact indicates that iron itself negatively impacts on hematopoiesis, and in some conditions removal of iron burden from the hematopoietic environment can restore normal hematopoiesis.

Deferasirox is easily absorbed in the gastrointestinal tract and has an elimination half-life of 8–16 h, which means that deferasirox is continuously present in the plasma with once-daily dosing [9]. In a large Phase III trial, deferasirox was comparable with DFO at decreasing iron burden in β-thalassemic patients [10]. Deferasirox also reduced iron burden in patients with various anemias including MDS [11]. These findings indicate that oral iron chelators can improve patients' quality of life by ameliorating organ dysfunction and preventing iron damage, even improving hematopoiesis itself. Oral iron chelators are expected to prolong survival of transfusion-dependent patients.

4 Japanese guidelines for the treatment of iron overload in transfusion-dependent patients

The clinical significance of iron chelation is undeniable and requires attention. With the availability of deferasirox in Japan, the frequency of continuous treatment may be strengthened and many more patients can benefit from chelation therapy. To help optimal iron chelation therapy, the National Research Group on Idiopathic Bone Marrow Failure Syndromes drew up the Japanese guidelines for the treatment of transfusion-induced iron overload. To date, guidelines for iron overload have been developed in several countries [6, 12–14], and the Japanese guidelines were designed to align with the international guidelines (see the paper by Dr. Gattermann in this issue). The essential features of the Japanese guidelines are depicted in Fig. 3 and Table 2.

The contents of the guidelines are as follows:

Patients who may benefit from chelation therapy: The guidelines are applicable to transfusion-dependent patients with primary (MDS, AA, PRCA, MF, etc.) and secondary (chemotherapy-induced, etc.) bone marrow failure. Transfusion-dependent patients are defined as those receiving >2 RBC units/month for ≥6 months. Because organ dysfunction becomes symptomatic after a certain period of time, it is suggested that iron chelation therapy is offered to patients with an expected survival of more than 1 year. The international guidelines for MDS patients also recommend that they should have a life expectancy of ≥1 year.

Diagnosis of iron overload: After patients become transfusion dependent, regular examination of serum ferritin is required to monitor iron burden at least once every 3 months. For early diagnosis of organ dysfunction,

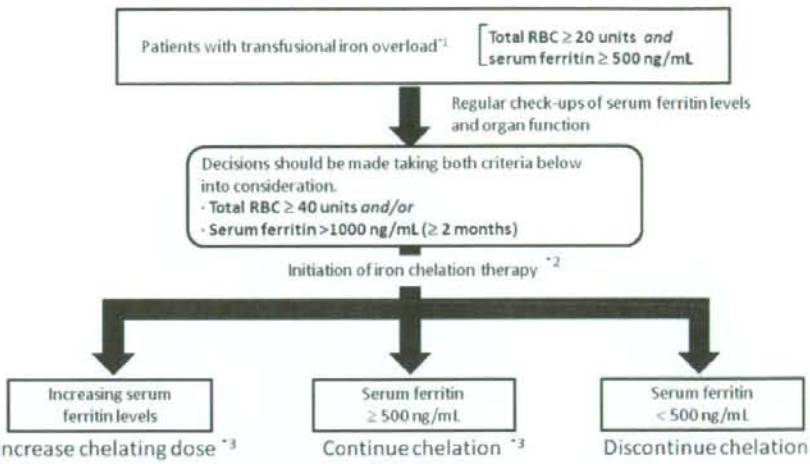

Fig. 3 A flow chart for the treatment of transfusion-dependent iron overload

*1 Patients who are transfusion dependent (≥ 2 RBC units/month for ≥ 6 months) and are expected to survive for >1 year.
*2 Monitoring serum ferritin levels at least once in 3 months is required.
*3 Regular check-ups of renal and hepatic function, and annual eye and hearing tests are necessary.

periodic check-ups of cardiac, hepatic and pancreatic endocrine functions are recommended.

Patients can be said to be iron overloaded when their serum ferritin levels reach >500 ng/mL and when they have received >20 Japanese RBC units (in pediatric patients, >50 mL/kg body weight). Severity of iron overload is determined by serum ferritin levels and organ dysfunction (Table 2, lower part).

Initiating iron chelation therapy: Administration of an iron chelator is the only recommended treatment for iron overload in patients with bone marrow failure. To initiate iron chelation therapy, confirmation of serum ferritin levels >1,000 ng/mL for more than 2 months, at least in two successive examinations, is recommended. The nationwide survey reported that more than 90% of patients who suffered from organ dysfunction had serum ferritin levels >1,000 ng/mL, and prevalence of hepatic dysfunction increases in parallel with ferritin levels [3] (Fig. 2). Therefore, a serum ferritin level >1,000 ng/mL is considered the appropriate point to initiate iron chelation. However, serum ferritin levels are not reliable in patients with inflammatory conditions such as Still's disease and hemophagocytic syndrome, or in those with malignancies. In these cases, transfusion history should be taken into account. Therefore, receiving a total of more than 40 Japanese RBC transfusion units (in pediatric patients, >100 mL/kg body weight) was included as another recommended criterion. As mentioned previously, about 75% of patients who received >40 RBC units have serum ferritin levels >1,000 ng/mL, indicating that 40 units of RBC transfusion can be a good indicator of transfusion-induced hyperferritinemia. However, transfusion history alone is also not reliable, because serum ferritin levels may not increase in patients with chronic bleeding and hemolysis. Furthermore, patients who have already discontinued transfusion therapy with successful treatment may not require iron chelation therapy. If neither of these two criteria is applicable, chelation therapy should not be started.

Target ferritin maintenance levels and adverse effects of iron chelators: During chelation therapy, monitoring of iron burden and organ functions should be continued. After initiating chelation therapy, serum ferritin levels should decrease, but if they continue to increase, even 3–6 months after starting treatment, an increase in dose is necessary. When patients are minimally transfusion dependent (<2 RBC units/month) or already free of transfusions, dose adjustment must be determined carefully.

It is recommended that serum ferritin levels are maintained at 500–1,000 ng/mL, and when ferritin levels are below 500 ng/mL at two successive examinations, chelators should be discontinued. As an excessive reduction in iron burden is harmful, the guidelines have determined this target value (500–1,000 ng/mL) with a safety margin.

As iron chelating agents can induce adverse effects on the kidney, liver and sensory organs [10], regular examination of renal and hepatic functions, and periodical (prior to treatment and annually after initiation) ophthalmologic examinations and hearing tests, are recommended. If an abnormal increase in serum creatinine level is noticed, the drug should be decreased or discontinued. In patients with a high risk of renal dysfunction, weekly monitoring of creatinine level is recommended, at least during the first

Table 2 Japanese guidelines for transfusional iron overload (main points)

Patients	Transfusion-dependent patients with bone marrow failure syndromes who are likely to survive for >1 year
Diagnosis of iron overload	1. Total RBC >20 units[a] (in pediatric patients, RBCs >50 mL/kg body weight) *and* 2. Serum ferritin >500 ng/mL
Criteria for initiating chelation therapy	1. Total RBC >40 units[a] (in pediatric patients, RBCs >100 mL/kg body weight) *and/or* 2. Serum ferritin >1,000 ng/mL Decisions should be made taking both criteria into consideration, especially for patients: –with chronic bleeding or hemolysis; –who no longer need RBC transfusions; –with complications that chronically raise serum ferritin levels independently of transfusion; e.g., Still's disease, hemophagocytic syndrome and malignancies
Target serum ferritin maintenance level	Serum ferritin 500–1,000 ng/mL

Classified severity of iron overload

Serum ferritin (ng/mL)	With normal organ function	With organ dysfunction
>500	Stage 1A	Stage 1B
>1,000	Stage 2A	Stage 2B
>2,500	Stage 3A	Stage 3B
>5,000	Stage 4A	Stage 4B

The severity of iron overload is defined by serum ferritin level and organ dysfunction (cardiac, liver and pancreatic endocrine dysfunction). The dysfunction must be considered to be related to iron overload; i.e., the organ dysfunction progresses as serum ferritin or transfusion burden increase

The criteria for specific organ dysfunction are as follows

–Cardiac dysfunction: LVEF <50%

–Hepatic dysfunction: abnormal transaminase levels, fibrosis and cirrhosis of the liver

–Pancreatic endocrine dysfunction: impaired glucose tolerance

[a] 20 and 40 units of the Japanese RBC transfusion correspond to 10 and 20 Western RBC units, respectively

month. Furthermore, if drug-induced hepatic injury is suspected, withdrawal of the drug with appropriate treatments is needed. It has been reported that iron chelators can cause hearing loss and cataracts. Therefore, if any signs of dysfunction are noticed a dose reduction or discontinuation of the drug is necessary and prompt consultation by an ophthalmologist or otorhinolaryngologist is required. In pediatric patients, annual monitoring of height, weight and state of secondary sex characteristics are needed for an early diagnosis of abnormal development.

5 Conclusions

The retrospective survey of transfusion-dependent patients revealed that the mortality rate is raised in heavily iron-overloaded patients, with liver and cardiac dysfunction being the primary cause of death [3]. Daily or continuous chelation therapy is effective in reducing iron burden and improving organ function, but practically, daily or continuous administration through parenteral injection is difficult. In Japan, a novel oral chelator, deferasirox, has recently been approved. Oral iron chelators can improve compliance of treatment and many more patients who need iron chelation may benefit from a reduction in iron burden and improvement of organ function, which ultimately may lead to the improvement of patients' prognosis and quality of life.

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