Abstract. A combined deferasirox (DFX) and deferiprone (DFP) treatment protocol for relieving thalassemia patients’ iron-overload was designed and the pharmacokinetic study was performed by LC-MS/MS. For this open-label, randomized trial, eight patients were recruited and randomly allocated to different treatment regimens: (A) monotherapy with single oral dose of DFX 30 mg/kg, (B) monotherapy with DFP 80 mg/kg/day, twice daily, (C) combined therapy with DFX and DFP (DFX 30 mg/kg for first dose, DFP 40 mg/kg 7 hours later, and DFP 40 mg/kg after another 7 h) and (D) concurrent therapy with DFX 30 mg/kg and DFP 80 mg/kg. Descriptive statistics evaluated pharmacokinetic parameters, AUC0-t, AUC0-inf, Cmax, Tmax, T1/2 and MRT. A positive pharmacokinetic drug interaction was observed in combined therapy. In case of DFX, combined therapy tallied about 2-fold larger than monotherapy in AUC and Cmax, 1.5-fold larger in Cmax, 1 hour longer in Tmax, but 1 hour shorter in T1/2. Regarding DFP, most such parameters of combined therapy concurred with monotherapy. Conversely, negative drug interaction was observed in concurrent therapy. With DFX, concurrent therapy attained 1.2- to 2.2-fold lower than monotherapy in AUC0-t and Cmax, 0.6-h shorter in Tmax, and 3-fold longer in T1/2. With DFP, concurrent therapy proved approximately 2-fold larger than monotherapy in AUC and Cmax, 2.5-fold longer in T1/2, and 1.4-fold longer in MRT. Follow-up of subjects’ clinical examinations and subjective symptoms showed no adverse events. Our findings showed the combined therapy had advantages, safe, convenient and painless for patients, over the existing concurrent therapy with deferoxamine (DFO) and DFX.

Beta-thalassaemias are a group of inherited blood disorders caused by reduced or absent synthesis of beta chains of hemoglobin, resulting in variable genotypes ranging from severe anemia to clinically-asymptomatic individuals. Approximately 1.7% of the world’s population has the alpha- or beta-thalassaemia trait. Thalassaemia affects men and women equally and occurs in approximately 4.4 of every 10,000 live births (1). Iron chelators are needed to prevent damage to the heart, liver and endocrine glands from iron overload in patients with refractory anemia who receive regular blood transfusions. DFO has been the first-line iron chelating drug for treating transfusional iron overload for more than 40 years, although administered by subcutaneous infusion and leading to therapeutic non-compliance. Poor compliance negatively correlates with survival (2); novel advances in oral iron chelation therapy have become the highlight of thalassaemia management across these past two decades (3). DFX and DFP, because of their low molecular weights, are developed for oral administration to reduce iron overload as well as improve patients’ compliance and quality of life (4).

DFP (L1, CP20, Ferripox and Kelfer) is the first oral iron chelator clinically used to treat thalassaemia (5). Recent data suggest it is as efficient as DFO at protecting heart from iron overload (6, 7), which it has successfully chelated in many cases at a dose of 50-100 mg/kg/day (8). The agent is
available in liquid and tablet formulation, administered three
times daily at a recommendation dose of 75 mg/kg/day, not
above 100 mg/kg/day (9, 10). After oral administration, DFP
is rapidly absorbed by the gastro-intestinal tract. Peak
concentration in plasma occurs after about 45–60 min in
fasting patients and may be prolonged in fed patients. The
ingestion of food affected significantly only peak serum
concentrations, while total area under the curve and the
elimination half-life were unchanged. Mean elimination half-
life is reported as 1.5–2.6 h (11-13). The water-soluble DFP-
iron complexes with a ratio of 3:1 are formed and excreted in
urine (14).

DFX (ICL670, Exjade) is a potent and tridentate oral iron
chelator that binds iron in 2:1 ratio and subsequently
eliminated by fecal excretion. Absolute bioavailability of
DFX in the form of dispersible tablet is 70%. Plasma half-
life of DFX is long (8 to 16 h), compared to DFP, allowing
once-daily administration. The recommended initial dose is
20 mg/kg orally, once daily. Dosage can be adjusted to
30–40 mg/kg by monitoring patients’ serum ferritin levels;
treatment at higher dose of 30–40 mg/kg/day, compared to
lower dose, is not associated with unexpected changes in
safety parameters (15-17).

However, neither DFX nor DFP could remove the iron
overload totally from the body of thalassaemia patients.
Without adequate iron chelation therapy, almost all patients
accumulate potentially fatal iron levels toxic to the heart, liver
and endocrine glands, and consequently lead to organ
dysfunction. Several clinical studies indicate combined
therapy with one oral chelator and DFO offering benefits:
reducing cardiac or liver iron overload and serum ferritin
along with myocardial siderosis, improving cardiac function,
reversing and preventing endocrine complications, reducing
cardiac mortality, and improving survival (17). Combined
therapy, thus, merits design as standard treatment for severe
iron overload. To date, available combination therapy is one
oral chelator combined with DFO (18). Over time, the
parenteral administration of DFO may result in labile
compliance of patients. Few studies deal with effective
combination of two oral chelators. Voskaridou et al. and
Vasilios et al. demonstrated successful chelation with two
available oral chelators, DFP and DFX, in four patients,
especially on cardiac iron (19, 20). We intend to increase the
subject numbers and design several treatment regimens in the
present study to compare the pharmacokinetic differences.
Herein, we report on eight Chinese thalassaemia patients
receiving four designed treatment regimens, followed by
measuring drug levels of DFX and DFP in patients’ plasma
using LC-MS/MS. Through pharmacokinetic evaluation, an
effective, convenient, and high-compliance treatment regimen
may be recommended.

Patients and Methods

Patients. We recruited eight beta-thalassaemia patients from China
Medical University Hospital (CMUH) in Taiwan. Their mean age was
22.6±3.5 years (range: 18–28 years), mean height 161.1±6.0 cm
(range: 154.5–169.7 cm), mean weight 50.7±7.8 kg (range: 38.0–61.6
kg). Study staff explained the purpose of the trial and obtained
patients’ written informed consent before enrollment. This trial was
approved by the Institutional Review Board (IRB) of CMUH. Prior
to study, all participants reported to the study Center and fasted
overnight for a minimum of 10 h before dosing and were randomly
assigned to one of two groups receiving designed treatment regimens.

Study design. The controlled, open-label and randomized study was
conducted at CMUH and the study design was illustrated as Figure 1.
Eight subjects were divided into two groups. One male and five
females, were randomly allocated to Group 1 and received regimen
A. DFX (Exjade, Novartis Pharma; 125 mg, 250 mg, and 500 mg
tablet) were fully disintegrated in 200 mL drinking water with plastic
stirrer, dispersion swallowed within 10 min. Plastic drinking cup and
stirrer were rinsed with 50 mL drinking water. Additional dispersion was also consumed by patients. Through a venous catheter, serial blood samples (1 mL/each sampling) were collected in heparinized glass vacutainers at time 0 (pre-dosing) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 12 and 24 h after dosing. After 3-day washout period, Group 1 received regimen B. DFP (Kelfer, CIPLA, India) at 500-mg cap, were prepared and divided equally into two doses. The first dose was administered at 2:30 p.m., followed by the second dose at 9:30 p.m. Serial blood samples (1 mL/each sampling) were collected at Time 0 (pre-dosing) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7 and 16 h after the second dose. After another 3-day washout interval, Group 1 received regimen C. Serial blood samples were collected as described above for DFX study after the first dose and for DFP study after the second dose. Group 2 consisted of one male and one female undergoing regimen D. Blood samples (1 mL/each sampling) were collected at Time 0 (pre-dosing) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9, 10, 14, 24 and 36 hours after dosing. All samples were centrifuged, plasma collected and frozen at −20°C until analysis.

**Analytic assay.** Plasma concentrations of DFX and DFP were measured by LC/MS/MS. The validated method for analysis of DFX was conducted via modified method described previously (21), and for DFP established by our team (22). Procedures involved a simple protein precipitation with acetonitrile and purification by reverse-phase HPLC system. Detection was performed by positive ion electrospray ionization in multiple reaction monitoring mode for quantification of drugs and internal standard.

**DFX.** The standard calibration curves showed good linearity within a range of 0.2-80 μg/mL. The lower limit of quantification (LLOQ) was 0.2 μg/mL. The between-run (n=6) validation for nominal concentrations (0.3, 5.0, 60.0 μg/mL) was -1.8% to 1.0% (relative error) with precision (coefficient of variation) of 4.4-7.7%.

**DFP.** The standard calibration curves showed good linearity within a range of 0.1-20 μg/mL. The LLOQ was 0.1 μg/mL; between-run (n=6) validation for nominal concentrations (0.2, 1.0, 15.0 μg/mL) was -5.3 to 0.3% (relative error) with precision (coefficient of variation) of 4.6-7.3%.

**Pharmacokinetic assessments.** DFX/DFP plasma concentration-time profiles were analyzed by non-compartment method using WinNonlin Professional software, version 2.0 (Pharsight Corporation, USA). Peak drug concentration in plasma (Cmax) and time of peak drug concentration (Tmax) were obtained directly from the observed data. Area under the plasma drug concentration-time curve from time zero to time t (AUC0→t) where t is last time point with measurable concentration of compound in question, was derived by the linear trapezoidal rule. AUC from time zero to infinity (AUC0→∞) was determined as AUC0→t + Clast/Kel, where Clast is the last measurable concentration. Calculation of pharmacokinetic parameters was performed for the compound of interest via drug concentration data. Elimination half-life (T1/2) were calculated with ln 2/Kel, where the Kel is terminal elimination rate constant, obtained from log linear regression of plasma drug concentration-time data in terminal post-distributive phase. Mean residence time (MRT) equals total area under first-moment curve divided by AUC0→t. Descriptive statistics summarized pharmacokinetic parameters. Arithmetic mean and SD are reported for Cmax, AUC0→inf, T1/2, and Tmax.

**Results**

All subjects completed the study. Group 1 completed regimens A, B and C with 3-day washout interval. Group 2 completed regimen D. Tables I and II plot pharmacokinetic parameters of DFX and DFP: those of DFX for regimen C, except T1/2 and MRT, are significantly higher than that of regimens A and D. AUC0→t, AUC0→inf, Cmax, Tmax and MRT of regimen C, in contrast to regimen A, are higher by 89.4, 85.1, 44.2, 32.4 and 2.4%, respectively, T1/2 lower by 10.5%. Comparing regimens C and D, we find AUC0→t, AUC0→inf, Cmax and Tmax of regimen C higher than with regimen D by 135.8, 55.0, 224.3 and 60.7% but T1/2 and MRT lower by 68.6% and 42.3%, respectively. As for regimens D versus A, AUC0→t, AUC0→inf, Cmax and Tmax of regimen C higher than with regimen D by 135.8, 55.0, 224.3 and 60.7% but T1/2 and MRT lower by 68.6% and 42.3%, respectively. Table 2 illustrates mean plasma concentration-time profile of DFX: order of mean plasma concentration value among protocols is C > A > D. The results above indicate combined therapy as suggested protocol for

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**Table I. Pharmacokinetic parameters of DFX in regimens A, C and D.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Regimen A mean±SD (N=6)</th>
<th>Regimen C mean±SD (N=6)</th>
<th>Regimen D mean±SD (N=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0→t, μg•h/ml</td>
<td>288.7±144.4</td>
<td>546.9±319.2</td>
<td>231.9±97.1</td>
</tr>
<tr>
<td>AUC0→inf, μg•h/ml</td>
<td>361.7±211.0</td>
<td>669.6±490.4</td>
<td>431.9±228.4</td>
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<tr>
<td>Cmax, μg/ml</td>
<td>34.2±20.4</td>
<td>49.3±17.6</td>
<td>15.2±2.8</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>3.4±0.7</td>
<td>4.5±0.6</td>
<td>2.8±2.5</td>
</tr>
<tr>
<td>T1/2, h</td>
<td>9.5±3.8</td>
<td>8.5±4.3</td>
<td>27.1±7.8</td>
</tr>
<tr>
<td>MRT, h</td>
<td>8.4±1.2</td>
<td>8.6±0.8</td>
<td>14.9±0.3</td>
</tr>
</tbody>
</table>

**Regimen A:** DFX 30 mg/kg, once daily; **Regimen C:** DFX 30 mg/kg, DFP 40 mg/kg, and DFP 40 mg/kg administered with 7-hour interval; **Regimen D:** DFX 30 mg/kg and DFP 80 mg/kg administered simultaneously.

**Table II. Pharmacokinetic parameters of DFP in regimens B, C and D.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Regimen B mean±SD (N=6)</th>
<th>Regimen C mean±SD (N=6)</th>
<th>Regimen D mean±SD (N=2)</th>
</tr>
</thead>
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<tr>
<td>AUC0→t, μg•h/ml</td>
<td>52.0±3.7</td>
<td>54.6±4.6</td>
<td>96.9±3.0</td>
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<td>AUC0→inf, μg•h/ml</td>
<td>53.5±4.0</td>
<td>55.6±5.5</td>
<td>98.9±3.2</td>
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<tr>
<td>Cmax, μg/ml</td>
<td>15.4±5.7</td>
<td>17.5±10.4</td>
<td>26.5±0.4</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>1.5±0.8</td>
<td>1.6±1.2</td>
<td>1.5±0.6</td>
</tr>
<tr>
<td>T1/2, h</td>
<td>3.3±0.3</td>
<td>3.2±0.5</td>
<td>8.4±1.2</td>
</tr>
<tr>
<td>MRT, h</td>
<td>3.8±0.8</td>
<td>3.4±0.7</td>
<td>5.2±0.3</td>
</tr>
</tbody>
</table>

**Regimen B:** DFP 80 mg/kg/day, twice daily; **Regimen C:** DFX 30 mg/kg, DFP 40 mg/kg, and DFP 40 mg/kg administered with 7-hour interval; **Regimen D:** DFX 30 mg/kg and DFP 80 mg/kg administered simultaneously.
patients to acquire good plasma concentration of DFX without significant change in MRT compared to monotherapy. Conversely, concurrent therapy failed to attain good plasma DFX concentration as combined therapy, raising T1/2 and MRT and possibly long-term drug function in the body.

As for DFP, pharmacokinetic parameters of regimen C closely resemble those of regimen B, albeit lower than with regimen D. As shown in Table II, AUC0-t, AUC0-inf, Cmax and Tmax of regimen C are slightly higher than those of regimen B by 4.6, 3.9, 13.6 and 6.7%, respectively, T1/2 and MRT of regimen C slightly lower by 3.0 and 10.5%, respectively. AUC0-t, AUC0-inf, Cmax, T1/2 and MRT of regimen C, in contrast to regimen D, are lower by 43.9, 43.8, 40.0%, 61.9 and 34.6%, respectively, but Tmax higher by 6.7%. While comparing regimen D with B, we found AUC0-t, AUC0-inf, Cmax, T1/2 and MRT of regimen D significantly higher than those of regimen B by 86.3%, 84.9%, 72.1%, 154.5% and 36.8%, respectively, yet Tmax was the same as with regimen B. Figure 3 illustrates mean plasma concentration-time profile of DFP. The order of mean plasma concentration value for DFP in different protocol is D > C ≈ A. In Table II and Figure 3, pharmacokinetics of DFP seem unaffected by DFX administered seven hours ago, those of DFP apparently affected by DFX administered simultaneously. In concurrent therapy, elimination of DFP is delayed and may adversely affect liver and kidneys via long-term use.

Discussion

Though prevalence of β-thalassaemia is not as high as that of hypertension or cancer, a certain number of people suffer from the disease in Taiwan and need better treatment to removing iron overload due to sustained blood transfusions. This is a small pilot study for treating the iron overload in β-thalassaemia patients. Key regimens, regimens C (combined therapy) and D (concurrent therapy), consist of identical daily doses of DFX 30 mg/kg and DFP 80 mg/kg but with different administration method. Two other regimens, regimens A (monotherapy with DFX) and B (monotherapy with DFP), are general treatments used clinically and taken as study references in this work. A positive pharmacokinetic drug interaction was observed in combined therapy. In the case of DFX, combined therapy got about 2-fold larger than monotherapy in AUC, 1.5-fold larger in Cmax, 1 h longer in Tmax, but 1 h shorter in T1/2. Regarding DFP, most such parameters of combined therapy concurred with monotherapy. Conversely, negative drug interaction was observed in concurrent therapy. With DFX, concurrent therapy attained 1.2-2.2-fold lower than monotherapy in AUC, 1.5-fold lower in Tmax, 0.6-h shorter in Tmax, and 3-fold longer in T1/2. DFP, concurrent therapy proved about 2-fold larger than monotherapy in AUC and Cmax, 2.5-fold longer in Tmax, and 1.4-fold longer in MRT. Follow-up of subjects’ clinical examinations and subjective symptoms showed no adverse events.

Findings lend support to combined therapy with oral iron chelators DFX and DFP as recommended treatment for thalassaemia major and severe iron overload, especially patients in poor compliance with combination therapy with DFP capsule and DFO injection. Our team is undertaking large-scale, long-term study to gauge subjects’ urinary and fecal iron excretion as well as tracking of adverse events in order to confirm the treatment of combined therapy with two oral chelators. Hopefully combined administration of DFX and DFP provides continuous and helpful iron-chelation and improves patients’ health.
Author Contributions
The work presented here was carried out in collaboration between all authors. Ching-Tien Peng designed the research. Ta-Shu Song, Yow-Wen Hsieh, Tai-Lin Chen, Hong-Zin Lee, Jing-Gung Chung and Mann-Jen Hour performed the research and analyzed data. Mann-Jen Hour wrote the manuscript. All authors have contributed to, seen and approved the manuscript.

Conflicts of Interest
The Authors declare no conflict of interest.

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