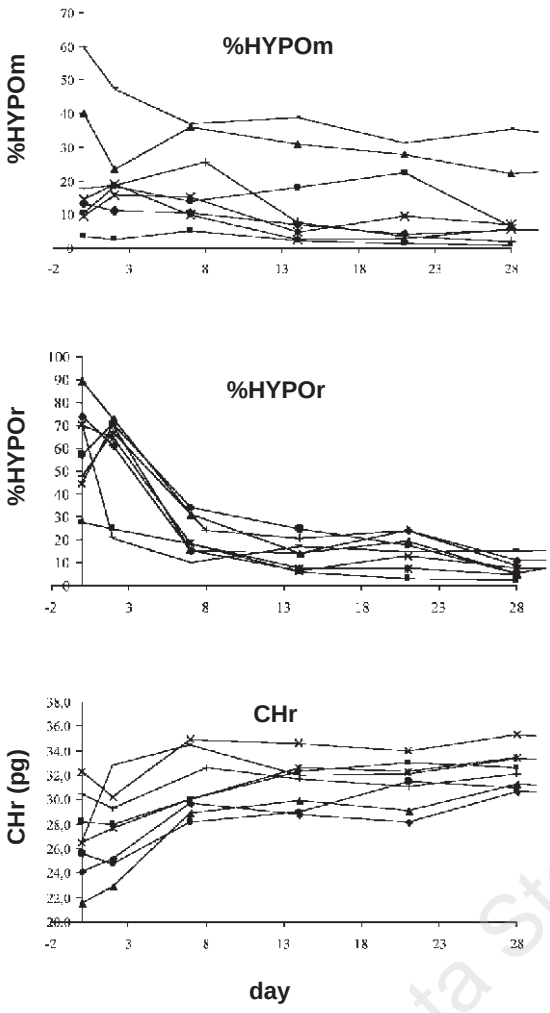

Figure 1. Figure 1. The responses of %HYPOm (top), %HYPOr (middle), and CHr (below) to oral iron medication in each of the iron defcient subjects (n=8). The samples were analysed on days 2, 7, 14, 21 and 28 after the start of iron medication.

and comparable to that of hemoglobin whereas the increase in CHr, and the fast decrease of %HYPOr could be observed already after 5 to 7 days. This suggests that CHr and particularly %HYPOr rapidly reflect increased iron availability for erythropoiesis and could be useful for monitoring the effectiveness of oral iron medication.

Sanna Kotisaari, Jarkko Romppanen,* Ulla Ågren,° Sakari Eskelinen,* Kari Punnonen**

**Department of Clinical Chemistry, Kuopio University Hospital, PL 1777, Kuopio; °Students' Health Service, Kuopio University, PL 1777, 70211 Kuopio, Finland*

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Correspondence: Kari Punnonen, M.D., Ph.D., Department of Clinical Chemistry and Hematology, Kuopio University Hospital, PL 1777, 70211 Kuopio, Finland. Fax: international +358.17.173200. E-mail: kari.punnonen@kuh.fi

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Combined therapy with desferrioxamine and deferiprone in thalassemic patients: effect on urinary iron excretion

Desferrioxamine B mesylate (DFO) and deferiprone (1,2-dimethyl-3-hydroxypyrid-4-one) (DFP) have been used for the treatment of hemosiderosis in patients with thalassemia major.[1] Preliminary studies have suggested that chelation is enhanced by the simultaneous administration of DFO and DFP.[2-6] In this study we evaluated the urinary iron excretion (UIE) of patients during treatment with DFO or DFP or the two drugs simultaneously.

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Sixty patients (32 females) with transfusion-dependent β-thalassemia major, aged 16 -37.7 years (mean: 24±4.5), were included in the study. Their ferritin levels ranged between 512 and 9359 μg/L (mean: 3118±1861). Nine patients were splenectomized. Eleven had anti-hepatitis C antibodies. All patients had been on regular chelation therapy with either DFO

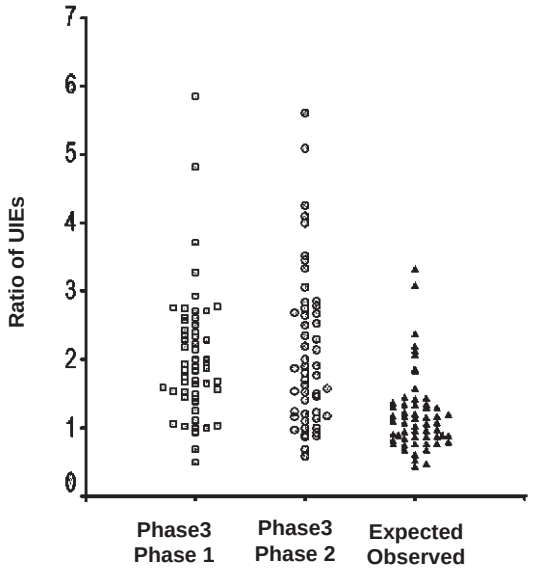

Figure 1. Dot plots of the ratios of UIE of combined therapy of DFO with 2 doses of DFP to UIE during treatment with either DFO (phase 3/phase 1, ❐) or DFP (phase 3/phase 2, ❍) and of the ratios of the observed UIEs to the expected UIE during treatment with combined therapy of DFO with 2 doses of DFP (phase 3, ▲).

(51 patients) or DFP (9 patients). Daily iron accumulation from transfusions was estimated based on the total amount of transfused packed red blood cells in the year prior to the study.

The study was approved by the appropriate institutional review committee. It included 4 phases, each one lasting 2 weeks. During phase 1 treatment consisted of DFO (Desferal, Novartis, Switzerland) at the dose which the patients had last used (mean: 40.8±6.2mg/kg/day). During phase 2, patients received DFP (Ferriprox™, Apotex, Canada). During phases 3 and 4 they received combined therapy with DFO and DFP (Table 1).

Four measurements of daily urinary iron excretion (UIE) were performed during each phase and the mean was calculated. UIE was measured by atomic absorption spectroscopy. The expected UIE was defined as the expected result of the interaction of the two chelators with the assumption that their effect is additive.

The significance of results was evaluated by the Wilcoxon signed-ranks test, the Wilcoxon rank sum or Spearman's correlation coefficient r_s.

The UIE was similar in phases 1 and 2 (monotherapy). Both combination regimens resulted in higher UIE than monotherapy with either DFO or DFP ($p<0.001$) (Table 1). On an individual basis, the combined therapy in phase 3 resulted in a 1.8-fold and 1.9-fold median increase in UIE compared to monotherapy with DFO and DFP, respectively (Figure 1). The median ratio of the observed to the expected UIE of phases 3 and 4 was 1.0 (25^{th}-75^{th} percentile values: 0.85-1.34) and 0.99 (25^{th}-75^{th} percentile values: 0.76–1.32), respectively (Figure 1).

The UIE of DFP was related to ferritin ($r_s = 0.307$, $p = 0.007$). The magnitude of UIE increase after combined therapy compared to DFP ($r_s = -0.292$, $p = 0.026$) and the ratio of observed to expected UIE were inversely related to ferritin ($r_s = -0.276$, $p = 0.036$). These findings reflect the substantially high UIE with DFP observed in patients with significant hemosiderosis. No relationship was documented between UIE and gender, age, spleen or hepatitis C status.

Table 1. Treatment protocol and results.

	Phase 1	*Phase 2*	*Phase 3*	*Phase 4*
Treatment protocol				
DFO	30-55 mg/kg/day		30-55 mg/kg/day	30-55 mg/kg/day
DFP		25 mg/kg every 8 hours	2 doses of 25 mg/kg*	3 doses of 25 mg/kg*
UIE (mg/kg/day)				
median	0.28	0.30	0.54	0.65
25^{th}-75^{th} percentile	0.21-0.45	0.18-0.43	0.40–0.79	0.48-0.72
Iron balance° (mg/kg/day)				
median	0.18	0.19	-0.06	-0.12
25^{th}-75^{th} percentile	0.06 to 0.27	-0.03 to 0.32	0.28 to 0	-0.25 to -0.12
Positive iron balance (% of patients)	78.6	69.6	23.6	15.6

**The first dose of DFP was taken at the start, the second dose 2 hours before the end of the DFO infusion and the third dose 6-8 hours (during phase 4 only) after the end of the DFO infusion. °Iron balance is defined as: [daily iron accumulation from transfusions] – UIE. Possible fecal excretion, which has previously been estimated at 30-200% of UIE for DFO and 10%-20% of UIE for DFP, was not taken into account.*

Mean daily iron accumulation from transfusions was estimated at 0.48±0.08 mg/kg/day. Iron balance in the various phases is shown in Table 1.

DFO and DFP may have different tissue distributions. DFP, being a small lipophilic molecule, enter the cells more freely and may mobilize excess iron from the cells more efficiently.[2,7-8] The tissue-mobilized iron may then be transferred from DFP to DFO in the extracellular space, as DFO has a higher affinity for iron than does DFP. This synergistic interaction of DFP and DFO is the basic concept of the *shuttle* hypothesis, supported by clinical data and results from animal experiments.[6] We documented a significant variability in the interaction of the two chelators, confirming that their mode of interaction is complex, being additive in some patients and truly synergistic in others (i.e. with efficacy more than the sum of individual efficacies of each chelator). Although the *shuttle* phenomenon may indeed occur, it could be that the two agents just chelate iron from different pools or from the same pools but without interacting or competing.

Previous reports on UIE in response to DFO and DFP are in agreement with ours.[9,10] We were unable to demonstrate a difference in UIE between combined therapies of DFO with 2 or 3 doses of DFP. This finding, which may be due either to a small sample size or to a saturation effect of combined therapy, is important in designing long-term therapy, in which other fac-

tors such as toxicity and cost need to be considered.

With the current chelation regimen, the balance between iron accumulation and excretion is fine.[1,6,10] In contrast to the iron balance achieved by monotherapy with either DFP or DFO, iron balance achieved with combined therapy was negative in the majority of patients.

In conclusion, combined therapy with DFO and DFP showed an additive and occasionally synergistic effect on UIE, which could reach levels higher than iron accumulation from transfusions, leading to a negative iron balance. Long-term studies are required to validate the efficacy and safety of combined therapy.

Antonis Kattamis, Christina Kassou,* Helen Berdousi,* Vasilis Ladis,* Ioannis Papassotiriou,° Christos Kattamis**

**First Department of Pediatrics, University of Athens; °Department of Clinical Biochemistry, 'Aghia Sophia' Children's Hospital, Athens, Greece*

Correspondence: Antonis Kattamis, MD, First Department of Pediatrics, University of Athens, Medical School, 'Aghia Sophia' Children's Hospital, Thivon and Levadias, Goudi 115 27, Greece. Phone: international +30.2107467129. Fax: international +30. 2107759167. E-mail: ankatt@med.uoa.gr

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Lack of *Bcr-Abl* point mutations in chronic myeloid leukemia patients in chronic phase before imatinib treatment is not predictive of response

We describe the presence of abl point mutations detected using a highly sensitive technique in 5 out of 9 patients with chronic phase CML resistant to imatinib. These mutations were not detected in samples obtained before initiating therapy with imatinib. Unless more sensitive techniques are developed, the presence or absence of point mutations before starting imatinib therapy will not help in predicting responses to treatment.

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Despite the positive results of treatment with imatinib mesylate (IM) in patients with chronic myelogenous leukemia (CML)[1] a number of patients develop clinical resistance to this drug, resulting in progression of the disease at 18 months in 11% of interferon resistant/intolerant patients.[2] Most patients in blast crisis will eventually suffer disease progression despite continuous treatment with imatinib.[3,4]

Among the different mechanisms of *in vivo* resistance to IM, the most frequently detected in patients with advanced phase (accelerated or blast crisis) CML is point mutations in the kinase domain of *Abl*.[5-7] We studied the presence of *Bcr-Abl* mutations in a homogeneous group of CML patients in chronic phase with primary cytogenetic resistance to IM in order to determine the incidence of point mutations and whether the presence of these substitutions before treatment could predict resistance to IM therapy.

We studied a group of 89 patients with CML enrolled in an extended access trial of IM (chronic phase CML patients resistant to or intolerant of interferon-α). All patients had 100% Philadelphia positive metaphases. Patients with no cytogenetic response after at least 6 months of therapy were defined as having primary resistance to IM and analyzed for the presence of *Abl* mutations. Bone marrow mononuclear cells were obtained before initiating treatment with IM and every 3 months thereafter.

Total RNA was extracted using RNeasy®Mini Kit (Qiagen, Hilden, Germany) from frozen cells. Total RNA (1 μg) was used for cDNA synthesis using SuperScript™ II RNase H-RT (Invitrogen Life Technologies, Paisley, UK) with random hexamers. A BCR-ABL transcript of 1.3 kb was amplified by PCR using 4 μL of cDNA and CM10 (5´-GAAGCTTCTCCCTGACATCCGT-3´) and 3ABL2 (5´-CGGACTTGATGGAGAACTTG-3´) primers under the following conditions: 94°C for 10 min, 30 cycles at 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 90 seconds, and a final elongation cycle at 72ºC for 10 min. The Abl kinase domain was amplified in a second PCR using 1 μL of the first PCR product and 5ABLKD (5´-GCGCAACAAGCCCACTGTCTATGG-3´) and 3ABLKD (5´-GCCAGGCTCTCGGGTGCAGTCC-3´) primers with the following conditions: 94°C for 10 min, 30 cycles at 94°C for30 seconds, 70°C for 30 seconds and 72°C for 30 seconds, followed by an elongation cycle at 72°C for 10 min. All PCR reactions were carried out in a total volume of 25 μL, with 2.5 U of native PFU polymerase (Stratagene, Amsterdam, The Netherlands), 0.4 mM dNTPs and 20 pmol of each primer.

The second PCR product (597 bp) was subcloned into pCR® 4-