WHAT A HİGH FREQUENCY OF DEFERİPRONE-İNDUCED AGRANULOCYTOSİS İN A TURKİSH POPULATİON OF THALASSEMİA MAJOR

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Bir Türk talasemi majör hasta grubunda deferiprona bağlı agranülositozun yüksek sıklığı

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Özet

Deferipronun agranülositoz yapma potansiyeli mevcuttur. İskenderun Devlet Hastanesi Kalıtsal Kan Hastalıkları Merkezi ve Mustafa Kemal Üniversitesi Tıp Fakültesi Hematoloji Servisince takip edilen talasemi majör hastalarının kayıtları incelendi. Onsekiz vaka desferoksamin, beş vaka deferipron ve 21 vaka her ikisini de kullanmaktaydı. Tedavinin ilk yılında deferipron kullanan 26 hastadan üçünde (%11.5) agranülositoz gelişti. Bu oran, önceki yayınlarda belirtilen agranülositoz görülme oranı olan %0.5-1 ile karşılaştırıldığında, arada istatistiksel olarak anlamlı bir farkın mevcut olduğu görüldü (p<0.01). Hafif nötropeni hiçbir vakada görülmedi ve bu oran da anlamlı şekilde düşüktü (%0.0'a karşılık %8.5, p<0.01). Agranülositoz görülen vakaların üçü hem desferoksamin hem de deferipron kullanmaktaydı ve vakaların bir tanesi bayandı. Ayrıca, agranülositoz görülen vakaların ikisi splenektomiliydi ve hiçbir vakada antiHCV pozitifliği tespit edilmedi. Sonuç olarak, kalıtımsal faktörler ve desferoksamin ile birlikte kullanımın, deferiprona bağlı agranülositoz

için önemli risk faktörleri olduğu ve bayan cinsiyet, geçirilmiş splenektomi veya hepatit enfeksiyonlarının getirdiği risklerin düşük olabileceği düşünüldü.

Anahtar kelimeler: Talasemi majör, artmış demir yükü, desferoksamin, deferipron, ICL 670

Summary

Background: Deferiprone may cause agranulocytosis.

Methods: All cases of thalassemia major followed by Center of Hereditary Blood Disorders of the Iskenderun Public Hospital and Hematology Department of the Mustafa Kemal University were retrospectively reviewed.

Results: Eighteen cases were on desferrioxamine therapy, five on deferiprone, and 21 on both initially. Three of 26 deferiprone receiving cases developed agranulocytosis in the first year of therapy, so its frequency was 11.5%. When we compared the ratio with previous reports (0.5-1%), difference was significant (p<0.01). Milder neutropenia was observed in none of deferiprone receiving cases, which was significantly lower (0.0% vs 8.5%, p<0.01). Three agranulocytosis cases were both on desferrioxamine and deferiprone therapies, and only one case was female. Additionally, two of them were splenectomized, and none was antiHCV positive.

Conclusion: Hereditary factors and combination with desferrioxamine are probably the most important factors for higher frequency of deferiprone induced agranulocytosis rather than female sex, splenectomy operations, or hepatitis infections.

Key words: Thalassemia major, iron overload, desferrioxamine, deferiprone, ICL 670

Introduction

Thalassemia major (TM) represents itself as a severe anemia during early months of life, and regular red blood cell transfusions are necessary to prevent cardiac decompensation. The recommended transfusion scheme leads to the transfusion of 100-200 mL/kg/year of pure red cells, which is equivalent to 0.3-0.6 mg/kg/day of iron (1). When the iron-binding capacity of transferin and ferritin is exceeded, iron starts to generate harmful free radicals and causes multiorgan damage including endocrine organs, liver, and most importantly the heart. Additionally, there are two other large groups of patients requiring chelation therapy. In the first group, there are non-transfusion-dependent thalassemia intermedia cases in whom the iron absorption is about 5–10times of the normal and around 0.1 mg/kg/day. In the second group, there are regularly transfused patientswith sickle cell anemia, aplastic anemia, myelodysplasia, myelofibrosis,red cell aplasia, congenital dyserythropoieticanemia, and congenital sideroblastic anemia like disorders. Iron chelation therapy was associated with a significant decrease in the rate of complications and with a dramatic increase in survival, especially in the thalassemia cases.

Although liver iron has been described as the "gold standard" for determiningbody iron (2), serum ferritin is a much easier technique. However its serum level increases in, inflammations and infections and decreases in vitamin C deficiency. Liver iron capacity can only be measured via biopsy (which may be inaccurate due to fibrosis, cirrhosis, or unequal distribution of the iron) or by the superconductingquantum interface device or by magnetic resonance imaging (MRI).

International Thalassemia Federation guidelines recommend maintaining serum ferritin levels around 1000 µg/L (1). So chelation therapy is usually begun in children after 10–20transfusions. As the most widely used iron chelator, standard therapy with desferrioxamine (DFO) is initiated with a dose of 40 mg/kg/day infused subcutaneously over a period of 8–12 hours on 5–7 nights each week by using a battery-operated infusionpump. Alternative routesof administration include twice-daily bolussubcutaneous injections (3), continuous infusions over 24 or 48hours by using disposable prefilled balloons (4), and continuousintravenous infusion by using an indwelling central line or Portacath (5). Deferiprone, the orally active bidentate iron chelator, is now licensed for patientswith TM unable to be effectively treated with DFO. It forms a 3:1 chelator/iron complex mainly

excreted in the urine. Its iron chelation site is inactivatedby glucuronidation, the speed of which varies from patient topatient. This explains much of the individual variation in response (6). Deferiprone mobilizes iron from parenchymal and reticuloendothelial poolsand from transferrin, ferritin, and hemosiderin. Unlike DFO, it is also capable of chelating iron from intact red cells invitro and in vivo, shown in patients with sickle cell anemia (7)and thalassemia intermedia (8). The enhanced ability of deferiprone tocross cell membranes may underlie what is emerging as its superior ability to protect the heart andalso the "shuttle effect" for iron when the two drugs are givensimultaneously (9). So deferiprone enters into the cells and brings the iron into plasma, which is transferred to DFO for excretionin urine and feces. On the other hand, the continuous deferiprone with intermittent DFO therapy decreases the drug dosages, toxicities, and number of days of DFO infusion, so improves the compliance and quality of life. For example, glucose and protein loss associated with high dose of DFO therapy is resolved after combined use of the two chelators with lower doses (10). Nowadays, as another oral iron chelator ICL 670 was developed. Preclinical studies show that it forms a 2:1 chelator:iron complexand produces an increase predominantly in fecal iron excretion but it is not on marketing in Turkey, as in most part of the world, now.

Although deferiprone may cause transient gastrointestinal symptoms, abdominal pain, arthropathy, and transient elevations in serum transaminases (10), its serious side effect is agranulocytosis. The frequency of agranulocytosis was detected as 0.5% in a multicenter study involving 187 patients on long-term treatment with deferiprone alone (11), and reported as 0.5-1.0% in a review, recently (12). We tried to detect the frequency of deferiprone induced agranulocytosis in a Turkish population of patients with TM in the study.

Material and Methods

All of the cases with TM followed by the Center of Hereditary Blood Disorders of the Iskenderun Public Hospital and the Hematology Department of the Mustafa Kemal University between January 2005 and April 2007 were retrospectively reviewed. Criteria for exclusion from the study were severe liver and kidney diseases, pregnancy, or lactation. Chelation was initiated after one year of regular transfusions and/or 12 to 15 transfusions and/or when the serum ferritin level reached up to 1000 µg/L in patients. Chelation was initiated as desferrioxamine (DFO) and/or deferiprone. DFO was given with a dose of 25-35 mg/kg/day under the age of 5 years, 40 mg/kg/day until the completion of growth, and 50-60 mg/kg/day

in adults via subcutaneous infusions. It was given 2 to 7 days/week according to the serum ferritin level: 7 days per week when the ferritin level was greater than 5000 ng/mL, 4-5 days/week when the level was between 3000 and 5000 ng/mL. As the serum ferritin level decreased, the prescription was modified: DFO was suggested for 3-4 days/week when the ferritin level was between 2000 and 3000 ng/mL, and 2-3 days/week for values lower than 2000 ng/mL. So chelation therapy was tailored to the needs of the individual patients. Deferiprone was prescribed for the cases at or above the age of 6 years, either in DFO intolerant cases to decrease its infusion numbers peer week or in cases with insufficient decreases of serum ferritin with DFO alone, and it was initiated with a dose of 75 mg/kg/day in three divided administrations at least an hour before food orally (13). Almost all of the patients preferred to perform the DFO infusions at night and take deferiprone during the day. A complete blood count and white cell differential were obtained every 7 to 10 days by using an electronic cell counter (LH700-Beckman Coulter, Fullerton, CA). Absolute neutrophils was counted under microscope by excluding normoblasts. If the absolute neutrophil count fell below $0.5x10^9$/L, which is called as agranulocytosis, therapy with deferiprone was temporarily interrupted and the number was counted in the other day. If the count is found below $0.5x10^9$/L again, the deferiprone therapy was withdrawn permanently. If the neutrophil count was found between 0.5-$1.5x10^9$/L initially, called as milder neutropenia, it was repeated in the other day, and if it was detected greater than $1.0x10^9$/L, the drug was continued with a close follow up. If it was found between 0.5-$1.0x10^9$/L in the other day, the drug was withdrawn and waited for resolution and initiated again. But in cases with a neutrophil count below $0.5x10^9$/L in the other day, deferiprone was withdrawn and never started again. Alanine aminotransferase (ALT) levels were determined initially and monthly afterwards with standard methods. In cases with an ALT level greater than 10-fold of the upper normal limit, the drug was planned to be withdrawn transiently. Antibody against HCV (antiHCV) was searched by ELISA (Abbott AxSYM HCV, version 3.0, Wiesbaden, Germany) and HCV RNA by polymerase chain reaction (Roche Cobas Amplicor HCV Monitor Test, version 2.0, Indianapolis, IN) methods, and HCV RNA positivity was accepted as an indicator of HCV infection. Serum ferritin was measured on venous blood samples every 2 months by using an automated immunoassay system (IMMULITE 2000®, Siemens Healthcare Diagnostics, Deerfield, IL). Odiometry and ophthalmologic examination were performed initially and once a year thereafter.

Statistical Analysis

Frequencies of all of the side effects, particularly agranulocytosis, were detected. Comparison of proportions was used as the test of Chi-square for statistical analysis.

Results

Fifty cases (26 males/24 females) of TM were studied totally. Their mean ages were 13.8 ± 7.1 (1-35) years. Four cases, under the age of 3 years, were not started to be treated yet. Additionally, two of the remaining 46 cases did not continue the routine follow up with unknown reasons. Initially, 18 of the cases were on DFO therapy alone, 21 on DFO plus deferiprone, and five of them were on deferiprone therapy alone. But three of the 26 deferiprone-treated cases developed agranulocytosis in the first year of therapy, so its frequency was detected as 11.5%. When we compared the ratio with the previous reports (11,12), there was a statistically significant difference in between ($p<0.001$). The total deferiprone exposure was 49 patient years, with an average of 1.5±0.4 years per patient, and its mean dose was 73 mg/kg/day. Agranulocytosis was reversible once deferiprone was discontinued and it resolved within 2-9 days after granulocyte colony-stimulating factor administration, without any sign of infection. Interestingly, milder neutropenia was observed in none of the deferiprone-treated cases, which was significantly lower from the previous report (0.0% vs 8.5%, $p<0.001$) (11). All of the three agranulocytosis cases were both on DFO and deferiprone therapy. One of them were female and two were male. Additionally, two of the three agranulocytosis cases were splenectomized. There were three cases with antiHCV positivity in the absence of HCV RNA positivity. All of the three cases of agranulocytosis were negative for antiHCV. Percentages of patients with other side effects including elevated ALT levels, nausea, vomiting, abdominal pain, and arthropathy were summarized in Table 1. There was no patient dropped out of the study due to the side effects other than the agranulocytosis, and the gastrointestinal symptoms resolved without discontinuation of the treatments. Some patients reported improvement of gastrointestinal symptoms when deferiprone was taken on eatings. In other patients, the deferiprone dose was temporarily reduced and gradually increased again without recurrence of the gastrointestinal symptoms. Gastrointestinal symptoms and arthropathy resolved significantly after the first year of therapy. No patient died or developed clinical findings of congestive heart failure during the study period. There were three patients who developed insulin-dependentdiabetes mellitus. No patient developed clinical evidence of end-stageliver disease. In none of the cases,

arthropathy caused discontinuation of the deferiprone therapy. The knee joints were mainly affected and the clinical symptoms were of stiffness and crepitus.

Table 1. Adverse events in patients receiving deferiprone.

Adverse events	**Percentage of patients**
Agranulocytosis	11.5% (3)*
Milder neutropenia	0.0% (0)
Elevated ALT	15.3% (4)*
Nausea and vomiting	34.6% (9)*
Abdominal pain	15.3% (4)*
Arthropathy	3.8% (1)

*$p<0.01$

Discussion

Despite the chelation therapy is associated with a significant decrease in the rate of complications and with a dramatic increase in survival of the thalassemia cases, physicians must be aware of the side effects, especially the life threatening ones, of the chelators. For example, DFO increases risk of infections, especially with Yersinia and occasionally with Klebsiella, and thrombosis (12,14). Beside that, high-frequency hearing loss, deafness, and retinal damage withimpaired vision (e.g., night blindness) may occur when large doses of the drug are given to less severely iron-loaded patients,especially children, in whom growth retardation and skeletaldamage have also been reported (12). Generalized hypersensitivityis rare, but painful local reactions at the injection site are common. Although deferiprone probably increases the compliance and efficacy of the chelation therapy, it has also some side effects, too. They include gastrointestinal discomfort, arthropathy, transient fluctuations in ALT levels, zinc deficiency, milder neutropenia, and as the serious one agranulocytosis (15,16). Hepatic fibrosis has also been suggested in a small retrospective study to be a consequence of deferipronetherapy (17). Transient gastrointestinal symptoms, such as nause, vomiting, and abdominal pain, are the most frequently seen complaints among them. In general, these events are reported earlyin the first year of therapy and uncommonly thereafter (11). Joint problems were associated with deferiprone in15% of the patients (11) and rarely required discontinuation oftherapy as in our study. But unlike the gastrointestinal complaints,

joint symptoms may occur throughout the treatment period. Interestingly, in a large follow up of patients withthalassemia receiving DFO it was found that drug-related arthralgiaand myalgia was present in 13% of patients, which may suggest thatjoint problems may be related to the underlying disease or chelation therapy (18). A reported association between the joint symptoms and serumferritin levels during the first year of therapy with deferiprone (19) did not achieve statistical significance inthe analysis after 4 years in another study (11). Whereas, joint symptoms occurred in up to 33% of patients in an Indian trial and high doses of deferiprone and greater degrees of iron overload were the predisposing factors (20). Not any association between antinuclear factor or rheumatoid factor titres and the joint symptoms has been shown in this or the above previous study (11), and the mechanism of the damage, whether free radical generation or immunological, remains to be determined. Despite an initial increase in ALT levels, trend analyses showed no significant change in ALT level and no significantchange in the percentage of patients with ALT levels greaterthan twice the upper limit of the reference range over 4 years,regardless of hepatitis C status (11). These findings confirm the transient nature of the changes in ALT levels that has beenreported previously and show no evidence of a progressiveincrease over time (11). Eventually, most patients with the above sideeffects can usually continue with the drug often after a periodof withdrawal, and retreatment initially at a lower dose and reverting back to subcutaneous DFO is only seen in a limited number of cases. Similarly, no one stopped the deferiprone therapy for the above transient and mild side effects in our study, too.

Agranulocytosis as the most serious complication of deferiprone occurs in about 0.5-1% of patients (11,12) and appears to be idiosyncratic. Among 13 patients reviewed by Hoffbrand, nine were female, suggesting a possibly increased susceptibility in females as occurs with other idiosyncratic drug agranulocytosis (21). But data from our study did not confirm a relationship between agranulocytosis and female sex as in the idiosyncratic agranulocytosis caused by other drugs (22). Because two of the agranulocytosis cases were male and one female, here. Additionally, two of the three agranulocytosis cases were splenectomized, and antiHCV positivity detected in none of the cases with agranulocytosis, here. Patients with agranulocytosisshould be permanently withdrawn from therapy, although patients with milder neutropenia have successfullybeen reexposed to the drug. Although the milder neutropenia occurred in 8.5% of patients before (11), the frequency was 0.0% here ($p<0.001$). The significantly higher frequency of agranulocytosis andlower frequency of

milder neutropenia found in our study may bea result of rigid monitoring of blood counts weekly. But we think that hereditary factors and combination therapy with DFO are probably the most important factors for determining the higher frequency of agranulocytosis rather than the female sex, previous splenectomy operations, hepatitis infections of the cases, or nature of the underlying disease, since patients with myelodysplasia and Blackfan-Diamond anemia have also suffered from agranulocytosis in another study (23), but all recovered, and no evidence either for a toxic or an immune mediated mechanism has been established yet (6,24). All patients with agranulocytosis subsequently regained normalneutrophil counts here, too.

As a conclusion, hereditary factors and combination with DFO are probably the most important factors for determining the higher frequency of deferiprone induced agranulocytosis rather than the female sex, previous splenectomy operations, or hepatitis infections.

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