Labile Plasma Iron: The Need and the Answer

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Chronic diseases of iron overload have two distinct features: (i) plasma iron levels are abnormally high, and (ii) the sustained excess leads to accumulation of iron in various organs. In the liver, the primary iron-storage organ, it results in hepatic dysfunction, while in the pancreas and the heart it causes diabetes and cardiomyopathy respectively.

The principal therapeutic approaches are based on the use of iron chelators, which are designed to reduce the iron burden on the body by forming complexes with liver iron and eliminating it. The efficacy of chelation therapy is usually monitored by assays of overall iron parameters such as plasma ferritin and serum iron, as well as by more specific indicators via biopsies of the liver or other organs. Non-invasive techniques are based on magnetic susceptometry (using the SQUID device) and in recent years also on magnetic resonance imaging (MRI). However, little attention has been given to the nature and source of the iron that

circulates in the plasma and the interstitial fluids and is assumed to be the direct cause of tissue iron overload.

The circulating form of iron that accumulates in the tissues in patients with iron overload is known as non-transferrin-bound iron (NTBI). As the name implies, it is thought not to be derived from ferritin, whose plasma levels generally reflect not only iron stores but also other pathological conditions (e.g., inflammation) not directly related to iron overload. It appears primarily in patients in whom, as a result of frequent transfusions, the iron-binding capacity of transferrin is exceeded. However, NTBI also occurs in other conditions, in which the patient's transferrin stores might not yet be fully saturated with iron. While only a relatively small fraction of the circulating NTBI finds its way into the liver, heart, pancreas, and other organs, its excessive and unrestricted accumulation can cause severe damage there. This potentially deleterious NTBI fraction, which is referred to as labile plasma iron (LPI), includes redox-active forms that are amenable to chelation.

What are the chelation strategies that should be chosen in order to minimize the deleterious effects of iron overload on both hepatic and extrahepatic tissue? One current approach is based on the use of agents that specifically relieve the iron burden within the liver, thereby indirectly reducing the LPI. A second approach is aimed at preventing the LPI in iron-overloaded patients from entering the heart. This is done by maintaining a basal level of chelating activity in the plasma during most hours of the day. A third would employ chelators that permeate cardiac or pancreatic cells and eliminate the iron-related cytotoxic activity. Although the optimal strategy is one in which the above three elements

are combined, they have not yet been incorporated into existing chelators or therapeutic regimens, whose efficacy has so far been proven only for removal of iron from the liver.

The Need

Despite the success of iron chelation therapy in keeping iron-overloaded patients in a state of negative iron balance for more than 2 or 3 decades, many develop serious and often fatal heart conditions, which are attributed not infrequently to iron accumulation in the heart. The accepted view is that in spite of the ongoing treatment (in most cases overnight infusion with the iron chelator desferrioxamine (DFO)), exposure of the heart to the circulating LPI (which reemerges 1–2 hours after cessation of the nightly treatment) can, over time, lead to the critical accumulation of iron in cardiomyocytes (via the dimetal transporter DMT1 or L-type Ca^{2+} channels, or both). Since such accumulation seems to occur much less frequently, if at all, in patients receiving DFO on a 24-hourly basis, and since plasma DFO does not extract iron from transferrin, it is reasonable to assume that any chelation treatment that can prevent the formation of LPI will prevent or minimize the harmful effects of iron on extrahepatic tissues. The question then arises: can LPI monitoring be used to assess the long-term efficacy of iron chelation treatment, and if so, how?

The LPI component of NTBI appears primarily in patients with hemosiderosis, and whose transferrin saturation levels exceed 85%. Recent epidemiological studies indicate that this is the critical level above which cardiac complications are most likely to occur. The simplest explanation linking cardiac

complications to LPI is that exposure of the heart to labile iron is a cumulative process that is likely to occur primarily in patients who are not continuously chelated; or in other words, when chelation is intermittent the treatment leaves wide windows of exposure to labile iron. This raises another question: can evaluation of LPI in the course of chelation treatment for transfusional iron overload (for example, in patients with thalassemia major) provide a direct measure of treatment efficacy, and if so, can LPI values be correlated with the risk of iron accumulation in the heart? These questions are currently being addressed, with the aim of defining LPI levels that constitute risk factors, particularly for heart conditions. Recent studies also suggest that LPI measurement can provide a simple, non-invasive tool for optimizing individualized chelation treatment of chronic patients with existing drugs and established regimens, as well as for assessing new drugs and new treatment protocols.

The Answer

With the advent of a new high-throughput technology (FeROS™) devised for assessing LPI (Aferrix, Rehovot, Israel), more than 4,000 patients suffering from thalassemia, MDS, or hemochromatosis have been screened for LPI. This parameter, rather than NTBI, provides a direct measure of iron that is both labile and chelatable. It fulfils a diagnostic need that is not answered by other tests of plasma iron: transferrin saturation lacks usefulness as well as reliability at iron saturations exceeding 85%, while serum ferritin, which responds to changes in body iron burden is too slow to be useful in this setting. Thus, measurement of LPI is of relevance for both diagnosis and therapy, and can be applied immediately in a clinical setting, with periodic repetition for long-term surveillance and follow-up

(see below). LPI can also be evaluated in conjunction with a test of NTBI called DCI (directly- or DFO-chelatable iron in serum), which assays various forms of NTBI that are accessible to DFO, including iron-chelates formed in the plasma, such as L1-Fe and ICL-Fe (complexes of iron with the oral iron-chelating agents deferiprone (L1) and ICL670, respectively), thus providing a measure of iron mobilized from the tissues to the plasma. DCI values, if measured when most of the chelator has been cleared from the plasma (2 h or 8 h after administration of DFO or L1, respectively), are expected to parallel the values of LPI.

For clinical trials based on oral administration of L1 (with or without nightly subcutaneous or intravenous administration of DFO), the two NTBI measurements (LPI and DCI) should be carried out according to one of the three schedules set out below. Blood specimens are withdrawn from all patients, and serum is prepared (~0.5 ml of serum for each pair of measurements), and stored frozen. As a minimum regime requirement, we suggest that schedule 2 (see below) be applied at the beginning and end of the trial and schedule 3 during treatment. A preferable regime is based on schedule 2 for the duration of the trial. The optimal regime consists of schedule 1 at the beginning and end of the study and schedule 2 during treatment. Obviously, a demanding schedule will be contingent on obtaining Helsinki approval.

Schedule 1: At onset and at termination of treatment (complete daily profile): Two to ten specimens, withdrawn at 2-h to 12-h intervals, over a 24-h period (between 7 a.m. and midnight), with a washout period of 4 h after the last L1 intake and 2 h after DFO infusion):

a. to determine L1 efficacy: blood is withdrawn before L1 intake;

b. to determine DFO efficiency: blood is withdrawn before infusion;

c. for combination (sequential) treatment: L1 during the day, DFO at night; as for a + b.

Schedule 2: At onset and termination of treatment (two specimens/day; this schedule gives a partial profile, with assays providing only an approximate measure of L1 or DFO efficacy in reducing LPI levels over the day).

a. for L1: one withdrawal before the morning intake and one before the last dose of the day (4 hrs after the last dose of L1);

b. for DFO: one withdrawal before and one 2 h after the end of the daily infusion;

c. for combination (sequential) treatment: L1 during the day, DFO at night; as for a + b).

Schedule 3: During treatment, one withdrawal every 2 months or as often as required, in the morning before L1 intake and before DFO infusion.

Similar schedules can be designed for assessing ICL670 and other novel iron chelators.

Our laboratory at the Hebrew University has been carrying out several small exploratory studies. The work is done either on a collaborative basis, with support from shared grants (e.g. EEC Consortium on Iron and Hemochromatosis) or as

outside contracts (e.g. NIH Blood Transfusion Unit, Treatment of Hemochromatosis).

For clinical or research services on a large scale (requiring hundreds of samples), we refer the work to Aferrix Ltd. (info@aferrix.com), which is developing commercial assay kits for LPI and DCI and is currently providing analytical services.