

## **Rockwell Medical Technologies, Inc.**

### **Soluble Ferric Pyrophosphate (SFP)** *Physiological Iron Maintenance Therapy*

#### **About SFP**

SFP, Rockwell's lead renal drug, is designed to safely treat anemia caused by chronic kidney failure, with initial indication for End Stage Renal Disease (ESRD). SFP is delivered to the ESRD patient via dialysate during each dialysis treatment (3-times weekly), traveling directly to the bloodstream and thereby maintaining optimal iron balance, avoiding liver toxicity and decreasing associated nursing and pharmaceutical administration costs.

#### **SFP – What Makes It Unique**

- SFP is water-soluble, which enables slow infusion of bio-available iron via dialysate during the 4-hour dialysis session, resulting in safe iron delivery and significant reduction in the costs associated with administering IV-iron, such as nursing time, MD trending and the use of disposable needles and syringes.
- SFP is a simple iron salt comprising ferric iron tightly bound to pyrophosphate with the highest binding stability constant vs. other large polymeric iron-carbohydrate complexes used intravenously.
- SFP's pyrophosphate component physiologically already exists naturally in the human body and is the chelator used by the body to normally transport iron inside the cells,
- SFP's slow infusion therapy (small doses with every dialysis treatment) serves to deliver enough iron to off-set the average 5-6 mg iron loss occurring during a dialysis session, thereby maintaining iron balance for continued hemoglobin generation without overloading tissue stores with iron, resulting in maximum EPO utilization and most likely lowering EPO dose and cost.
- SFP provides "Physiological Iron Maintenance Therapy" – designed to maintain iron balance for more effective erythropoiesis or red blood cell formation, thereby promoting maintenance of hemoglobin in the optimum 11-12 gm/dL range.
- SFP's unique characteristic, wherein iron is tightly bound to pyrophosphate, prevents dissociation of complex in the circulation, thereby greatly minimizing release of free/labile/catalytically active iron and consequent inflammation/oxidative stress.
- SFP's pyrophosphate component specifically promotes binding to iron in the blood-stream to apo-transferrin, which allows for the delivery of SFP bio-available iron directly to the bone marrow, bypassing the RE system in the liver, spleen and lymph nodes and thereby mitigating toxicity.
- Since SFP iron delivery bypasses the liver, it should be possible for the first time, to safely deliver bio-available iron to patients suffering from inflammation and concomitant EPO resistance. An NIH sponsored study is currently ongoing to further examine oxidative stress/inflammatory markers in dialysis patients and the natural anti-oxidant benefits of pyrophosphate.
- SFP's pyrophosphate component is an extremely potent inhibitor of calcium deposition in soft tissues including blood vessels, which should mitigate vascular calcification, a condition that leads to stiffening of the arteries, hypertension and heart disease.

- SFP's safety, dosing and delivery profile confer additional advantages (*versus* polymeric IV iron complexes) in home-dialysis setting.
- SFP is the first and only candidate being considered by the US FDA as maintenance iron therapy in hemodialysis patients that will target prevention of iron deficiency (polymeric IV iron complexes are approved only for treatment of established iron deficiency).

### **A History of Parenteral Iron Compounds**

Over 125 years ago it was recognized that oral iron supplements were either not sufficient or not tolerated in a significant proportion of patients. Therefore, intramuscular or intravenous administration of iron salts such as ferrous sulfate, gluconate, ascorbate, etc, was attempted but it was quickly determined that administration of these compounds was highly toxic. This toxicity has been attributed to the labile iron content that leads to peripheral circulatory failure and oxidative damage.

Nature recognizes this inherent toxicity of iron, a trace element, by storing and sequestering iron in mammals in the form of ferritin, which is a large macromolecular iron-protein complex.

In order to synthesize safer iron compounds for parenteral administration, scientists used the structure of ferritin as a prototype to construct similarly complexed iron compounds. Iron was conjugated with albumin or carbohydrates (e.g. dextran), thereby shielding iron from direct contact or uptake by parenchymal cells. This has led to the development of numerous iron carbohydrate complexes of varying molecular sizes such as iron dextran, iron gluconate, iron sucrose, iron-chondroitin sulfate, iron polysaccharide and ferumoxytol.

The acute adverse reactions from IV iron administration such as nausea, vomiting, cramps, back pain, chest pain, and hypotension are likely attributable to free or labile iron liberated (Van Wyck, *J Am Soc Nephrol* 15:S107-S111, 2004). The labile iron fraction follows the sequence Ferrlecit>Venofer>InFeD, varying inversely with core radius and overall molecular weight (Van Wyck, *J Am Soc Nephrol* 15:S107-S111, 2004). Therefore, iron dextran (InFeD), is more stable than Venofer®, which is more stable than Ferrlecit®. However, the stability or association constant of iron for dextran is still only about 100,000. This implies that 1 out of 100,000 molecules of iron dextran can be unstable and release labile iron in the circulation, thereby causing cellular toxicity and oxidative stress induced by iron complexes of this class.

***The release of free iron (or lack thereof) is a critical issue for toxicity of parenteral iron. An iron compound is needed that releases negligible labile iron in the circulation and SFP addresses this critical and unmet need.***

Based on the thermodynamic stability constants, compared to the binding of iron to dextran in polymeric iron complexes, iron is about ten thousand trillion times more strongly bound to pyrophosphate in SFP (Gupta and Crumbliss, *J Lab Clin Med*, 136:371-378, 2000). Because of this tight binding, ferric pyrophosphate doesn't dissociate and become an electrolyte (unlike e.g. ferrous sulfate) and hence SFP is tasteless. Therefore, SFP is believed to release negligible amounts of free or catalytically active iron, resulting in less oxidative stress and inflammation, compared to IV iron.

### **Processing of IV Iron Complexes and their Toxicity in the Body**

Iron carbohydrate complexes, when introduced into the circulation, are recognized and handled by the body similar to introduction of foreign particulate matters such as carbon, silica or micro-organisms. Phagocytic cells, located primarily in the liver, spleen and lymph nodes, are scavengers that act as a first line of defense against bacteria or foreign substances. These scavengers take up particulate matter including the iron carbohydrate complexes. Theoretically, when this first line of defense has been overwhelmed in trying to handle the administered iron complexes, it may not be able to handle bacteria and other pathogens as effectively, thereby, predisposing to infections. The iron-carbohydrate complexes tend to persist in the

circulation for days to weeks because of the slow clearance by the phagocytic cells. While in the circulation, iron can potentially serve as a nutrient for the microorganisms, thereby predisposing to infection.

Phagocytic scavengers are most abundant in the liver, and consequently, administered iron complexes are to a great extent stored in the liver, wherein, they have potential to cause toxicity to the neighboring liver cells (hepatocytes). The risk of IV iron use in patients with liver disease, specifically hepatitis C is of grave concern and yet remains unaddressed by DOQI (Berns, *Seminars in Dialysis*, 2003). IV iron causes more oxidative stress in hemodialysis patients with hepatitis C than without (Kato et al, *Kidney Int*, 2003). In patients with chronic hepatitis C, with no other cause for iron overload, iron itself may be a cofactor in the development of liver injury and correlate with disease severity. This could explain the reduced anti-viral effect of interferon in patients with raised liver iron content, and the beneficial biochemical and histological findings after phlebotomy in order to deplete the liver iron content [Olynx, *Am J Gastroenterology*, 2002]. In 1999, nationwide prevalence of anti-HCV amongst hemodialysis patients was 8.9%, with some centers reporting prevalences >40% (CDC unpublished data, 2001). Other studies of hemodialysis patients in the United States have reported hepatitis C prevalence of 10%-36% among adults and 18.5% among children [[www.hcvadvocate.org/news/NewsUpdates\\_pdf/2.2\\_Conference\\_Reports/renal\\_agenda/Section3/alter.pdf](http://www.hcvadvocate.org/news/NewsUpdates_pdf/2.2_Conference_Reports/renal_agenda/Section3/alter.pdf)]. In Japan, 19% of chronic hemodialysis patients have hepatitis C [Nakayama, *J Am Soc Nephrol*, 2000]. Further studies are needed to determine the safety of SFP in hemodialysis patients with hepatitis C. Theoretically SFP should be safer in hemodialysis patients with hepatitis C since SFP does not transit through the liver.

In normal health, the body has created defense mechanisms to prevent build up of iron stores in the liver and elsewhere. Numerous studies [as reviewed by Kletzmayer et al, *Nephrol Dial Transplant*, 2002], have linked excessive storage of iron with liver damage, atherosclerosis, heart disease, infections and cancer. For example, if a human being consumes excessive amounts of iron in their diet, the body's natural defense mechanisms turn off iron absorption in order to prevent excessive iron accumulation. The administration of large doses of polymeric iron complexes directly into the circulation bypasses the same natural defense mechanisms, dumps iron into storage as reflected by an increase in ferritin levels, and has the potential to cause all the toxic effects enumerated above if this exposure is continued for prolonged periods of time. The NIH sponsored study of SFP versus Venofer® in hemodialysis patients is specifically examining the effect of these two iron compounds on oxidative stress and inflammation over a period of 9 months. All IV iron compounds including Venofer and Ferumoxytol are known to increase ferritin levels while SFP maintains adequate iron delivery without raising ferritin levels (Gupta et al, *Kidney Int*, 1999).

Vascular calcification is recognized to be a major cause of morbidity and mortality in dialysis patients by causing stiffening of the arteries and predisposing to heart attacks, strokes and gangrene. Administration of polymeric iron complex has been linked to enhanced vascular calcification, possibly, by depleting pyrophosphate, a most potent inhibitor of vascular calcification. IV iron is contraindicated in patients with serious manifestations of vascular calcification such as gangrene of the skin and underlying tissues (calciphylaxis). SFP is likely to be safer in patients with calciphylaxis since iron in SFP is tightly complexed to pyrophosphate and would not deplete endogenous pyrophosphate. Furthermore, SFP has the shortest half-life amongst all the parenteral iron compounds.

### **Mode of Action: SFP vs. IV Polymeric Iron Complexes**

SFP is a simple iron salt and not a large polymeric complex. SFP in the circulation is metabolized in a physiological manner, similar to the handling of dietary iron absorbed from the gut. SFP binds directly to apo-transferrin resulting in the formation of transferrin, the circulating iron carrier, which transports the iron in SFP to the bone marrow for hemoglobin generation. Unlike polymeric IV iron complexes, SFP does not require uptake and processing by the liver to make the iron available for formation of transferrin. In other words, unlike the polymeric iron complexes, the body does not recognize or handle SFP as a foreign, particulate matter but rather like dietary iron.

Furthermore, by virtue of its mode of action SFP is likely to be devoid of the adverse side effects for polymeric iron complexes such as:

1. acute adverse reactions from release of free, labile, or catalytically active iron
2. liver accumulation and toxicity
3. permissive effect on hepatitis C, and
4. enhancement of vascular calcification or calciphylaxis,

In addition, negligible release of free/labile/catalytically active iron should translate into less oxidative stress. Pyrophosphate is a well known natural anti-oxidant. Infusion of pyrophosphate and/or reduction in free iron may result in less oxidative stress and hence the trend for a decline in ferritin levels in patients dialyzed with SFP dialysate, while ferritin levels increase in patients receiving I.V. iron (Gupta et al, *Kidney Int*, 1999). The primary goal of the ongoing NIH sponsored SFP study is to compare oxidative stress induced by SFP *versus* Venofer®.

Pyrophosphate physiologically already exists naturally in our body and is the chelator [a molecule that tightly binds iron making it inactive just as EDTA binds calcium] used by the body to normally transport iron inside the cells. In contrast, the polymeric IV irons are handled by the body's defense mechanism like a foreign substance and sent to the liver to be first contained, and then gradually released into the circulation. Pyrophosphate is an extremely potent inhibitor of calcium deposition in soft tissues such as arteries (vascular calcification). Calcium deposition in arteries leads to stiffening of the arteries, leading to hypertension and heart disease.

### **SFP – Overcoming EPO Resistance and Improving Hemoglobin Thereby Reducing the need for EPO**

A target Hb of 11-12 gm/dL can be achieved in the majority of chronic hemodialysis patients on EPO doses of up to 150 units/kg/week however, about 15% of HD patients require a dose >450 units/kg/week despite abundant iron stores. The most common cause of relative 'EPO resistance' in the latter group is an underlying inflammatory state. Inflammation stimulates liver to produce hepcidin, which then blocks ferroportin, an iron export protein that is needed to export iron out of phagocytic cells and into the circulation. IV iron requires processing by phagocytic cells and is often ineffective in delivering iron to the bone marrow in EPO resistant patients with inflammation since egress of iron derived from polymeric iron complexes is blocked to a great extent. Recent studies in hemodialysis patients with concomitant inflammation have shown that up to 80% of iron in polymeric IV iron complexes is not bio-available, lying trapped in the RE system of liver, spleen and lymph nodes) (DRIVE study: Coyne et al, *J Am Soc Nephrol*, 2007).

SFP delivers iron directly to transferrin without the need for processing by phagocytes and has been shown to safely and effectively maintain Hb in 80% of patients over 6 months without any need for IV iron (Gupta, *et al*: Dialysate iron therapy: Infusion of soluble ferric pyrophosphate via the dialysate during hemodialysis. *Kidney Int*. 55:891-898, 1999). SFP, by its mode of action, is likely to overcome EPO resistance, thereby increasing hemoglobin and decreasing the need for EPO.

### **SFP – Unique Parenteral Iron Supplement**

Since the development of parenteral iron complexes dating back to the 1860's, SFP is the first and only simple iron salt shown to be clinically safe and effective when administered directly into the circulation. A 6-month FDA Phase II study concluded that the slow delivery of SFP into the circulation in hemodialysis patients by addition of only 12 microgram of SFP-derived iron per deciliter of dialysate, is safe and effective. Over 700 such clinical administrations have demonstrated no adverse reactions related to SFP. The lack of toxicity of SFP is also supported by toxicology studies in rats, rabbits and dogs. These studies, both clinical

and toxicologic have shown that SFP does not produce liver toxicity in any species (or any other target organ toxicity), even at doses that are well above the therapeutic dose.

### **SFP – Home Dialysis**

In the 1980's and early 1990's, home hemodialysis was frequently prescribed and is again attracting interest because of the development of patient friendly dialysis machines and the recognition that more frequent dialysis (5-6 days a week), called 'daily dialysis', may be an advantageous dialysis modality that may be associated with improved patient outcomes and quality of life. With 'daily dialysis', requirements for EPO, phosphate binders and blood pressure medications are reduced markedly but iron losses are increased, thereby increasing the need for iron. Self-administration of IV iron at home is considered too risky and patients on daily dialysis have to come to the dialysis center in order to receive IV iron. This imposes extra demands on the dialysis personnel including the nurses and interferes with the schedule of the patients receiving In-Center Dialysis. SFP should be an ideal supplement for home hemodialysis patients because it does not pose any medical risk and does not require any extra effort on the part of the patient. With SFP administration it is now possible, for the first time, to safely administer parenteral iron in the home setting without the need of a nurse.

### **Goal of SFP Iron therapy**

Infusion of pyrophosphate and/or reduction in free iron may result in less oxidative stress and hence the trend for a decline in ferritin levels in patients dialyzed with SFP dialysate in our previous FDA phase IIa study, while ferritin levels increase in patients receiving I.V. iron.

*Rockwell proposes that the goal of iron therapy is to maintain iron delivery to the red blood cell precursors in the bone marrow, without overloading tissue stores in liver, spleen or lymph nodes. This is the cardinal principle in physiological maintenance of iron balance in normal individuals, and it should be no different for dialysis patients if iron toxicity is to be avoided.*

### **Small, Slow Doses of SFP Every Dialysis Session vs. Large, Fast, Bolus Doses of Polymeric Iron Complexes by Infusion**

Hemodialysis patients lose about 5 mg of iron during a dialysis session because of bleeding from the dialysis shunt, trapping of blood in the extra-corporeal circuit (dialysis tubing and dialyzer) and blood draws. Ideally hemodialysis patients should receive regular administration of small doses of iron supplements to maintain iron balance, i.e. "**Physiological Iron Maintenance Therapy**". It is cumbersome to administer small doses of intravenous iron with every dialysis session but is easily achieved by administration of SFP via the dialysis solution.

The toxicity of iron is related to the dose and rate of infusion and large doses given rapidly can be toxic. When iron is not administered regularly, eventually almost all hemodialysis patients develop iron deficiency that requires mega-doses of polymeric iron complexes to bring the patient back into iron balance, the so called intermittent "iron repletion therapy" – the patient's iron is consistently out of balance and varies within and outside the optimal iron balance range.

In a recent retrospective study of 19,150 patients with no missing hemoglobin or covariate values survival analyses indicated that each 1 g/dL increase in the residual standard deviation was associated with a mortality hazard ratio of 1.33 (95% CI: 1.22 to 1.45) even after adjusting for multiple covariates (Yang et al, "Hemoglobin variability and mortality in ESRD" *J Am Soc Nephrol* 2007; 18: 3164-3170). These included age, duration of ESRD, average hemoglobin level, serum albumin, aspartate aminotransferase, calcium, intact parathyroid hormone level, iron level, and erythropoietin dose. The relationship between hemoglobin variability and mortality, measured by the residual standard deviation of hemoglobin, remained significant even after adjusting for absolute hemoglobin levels and trends over time, suggesting that hemoglobin

variability represents an important physiologic stress. Regular administration of SFP will likely maintain iron balance, prevent development of iron deficiency and thereby maintain Hb in the target range while minimizing Hb variability.

**Requirements of Personnel when Administering SFP (via the Dialysate) versus IV iron**

<b>Personnel</b>	<b>IV iron Requirements</b>	<b>SFP Requirements</b>
Nurse	<ul style="list-style-type: none"> <li>• Track when the dose is due</li> <li>• Look up dose</li> <li>• Draw the dose in the syringe</li> <li>• Carry the syringe and keep it at the bedside</li> <li>• Administer after the dialysis session</li> <li>• Watch for adverse reactions</li> <li>• Record the dose administered</li> </ul>	None
Anemia Manager	<ul style="list-style-type: none"> <li>• Watch iron parameters monthly</li> <li>• Make decision to administer or hold iron for next 4 weeks</li> <li>• Decides a dose for administration</li> </ul>	<ul style="list-style-type: none"> <li>• Same</li> <li>• Same</li> <li>• Not needed since there is a single standard optimal dose</li> </ul>

**Clinical Development of SFP**

A Phase IIb double-blind, multi-center, randomized, placebo-controlled, nine-month study consisting of up to 130 patients at multiple dialysis centers in the United States is currently in progress. The primary endpoints of the study are to evaluate the safety of SFP at varying dosage levels in patients undergoing hemodialysis and to determine the optimal concentration of SFP that will maintain iron balance within the target hemoglobin range. Secondary endpoints include evaluating efficacy for changes in hemoglobin levels over time, reticulocyte hemoglobin content, incidence of systemic infection episodes, and to quantify the amount of SFP (iron) transferred from the dialysate directly to the blood during the dialysis session. Data updates via the independent Data Safety Monitoring Board will be announced as information becomes available.

In addition, NIH-sponsored 9-month clinical study started in December 2007, to examine oxidative stress markers in dialysis patients receiving SFP vs. IV iron (Venofer). Clinical study design includes: 30 patients, two sites, randomized, controlled, to examine maintenance of hemoglobin, iron parameters, need for intravenous iron and oxidative stress markers in patients receiving SFP via dialysate vs. patients receiving conventional iron-free dialysate. Patients in both groups are eligible to receive intermittent intravenous (IV) iron sucrose per protocol if iron deficiency develops. Updates will be announced as information becomes available.