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Supplementation of Total Parenteral Nutrition Solutions with Ferrous Citrate

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ABSTRACT. Daily infusion of a total parenteral nutrition (TPN) formulation containing 1 liter of 5.5% Travasol provides less than 0.1 milligrams of iron. By comparison, a formulation which includes a liter of 10% Travamin provides 2 milligrams of iron per day. To meet iron requirements in patients infusing formulations containing Travasol, iron was added as ferrous citrate. In *in vitro* experiments, 74% of this iron was available to transferrin. In seven patients in whom *in vivo* availability was tested by red cell incorporation, the mean availability was 81%. Ferrous citrate is recommended as a safe, effective additive to TPN solutions for adult patients requiring iron supplements.

Total parenteral nutrition (TPN) was originally used in the short-term maintenance of patients unable to achieve caloric balance by the oral route. As TPN formulae became more sophisticated, it was shown that nutrition in these patients could be sustained over long periods,[1, 2] provided attention was paid to requirements of trace nutrients such as minerals.[3–5] Among these, iron may be singled out for special consideration.

Chronic inflammatory bowel disease is a frequent indication for TPN. Significant numbers of these patients have iron-deficient erythropoiesis, not only because of associated blood loss from the gastrointestinal tract but also because of the internal iron block associated with chronic inflammation.[6] Furthermore, even in those individuals who are capable of some intake by mouth, oral iron may be malabsorbed or aggravate bowel symptoms. Against this background, recommendations have been made to give TPN patients iron dextran, either intramuscularly or added to the TPN solutions.[7, 8] A potential drawback to this therapy is the fact that this iron only becomes available to transferrin after it has been freed from dextran during processing in the reticuloendothelial system. In inflammatory disease, the return of this iron to plasma transferrin and to the erythron is blocked. Furthermore, in view of the frequency of side effects and the potentially lethal nature of rare anaphylactoid reactions, iron dextran is not an ideal choice for patients on TPN, especially when they infuse their solutions at home.[9, 10] For these reasons, the alternative approach of adding an iron salt to TPN solutions was investigated.

MATERIALS AND METHODS

Materials

TPN formulations. Patients on the home parenteral nutrition program infuse 2 liters of nutrients during a 12-hr overnight period. The infusions consist of 1 liter of 60% dextrose, the necessary electrolytes, vitamins and minerals, and either 1 liter of protein hydrolysate (10% Travamin) or 1 liter of crystalline amino acids (5.5% Travasol). These formulations will be referred to as TPN PH and TPN CAA, respectively.

Iron fortification. Iron salts in solutions at neutral pH form polymeric oxyhydroxide aggregates that are incapable of binding specifically to plasma transferrin.[11] Because chelators suppress this polymerization,[12] iron was added to the TPN solutions as ferrous citrate with a 20:1 molar ratio of citrate to iron. Five-ml stock solutions containing 2.5, 5, and 10 mg iron were prepared by dissolving ferrous ammonium sulfate and citric acid in normal saline and adjusting the pH to 2.0. The solutions were stored, protected from light, at room temperature. Some of these stock solutions were prepared with a trace label of $^{59}FeSO_4$ for use in *in vivo* studies of red cell utilization of iron. Stock solutions containing a similar trace label were used in gel filtration experiments with an iron concentration of 75 μg/ml and the same molar ratio of citrate to iron.

Analytical Procedures

Iron content. Measurements were made using the ICSH plasma iron procedure.[13] In this method, iron in acidified solution is detected colorimetrically after addition of a chromogen, bathophenanthroline sulphate. The iron concentrations in TPN PH and TPN CAA were compared. The iron concentration in 10% Travamin was also measured.

Availability of iron already present in TPN PH. An aliquot of TPN PH was adjusted to a pH of 7 by the addition of aqueous sodium bicarbonate and then incubated with freshly drawn normal plasma. After measurement of plasma iron and unsaturated iron binding capacity (UIBC) by the ICSH procedure,[14] the mixture was passed through an Amicon Model 8MC ultrafilter equipped with a PM-10 membrane (nominal molecular weight cut-off = 10,000 daltons). The availability of the

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iron was estimated from the change in plasma iron and UIBC. The ultrafiltration step was included because initial studies showed that TPN PH contained filterable iron binding components that interfered with the UIBC assay by providing a non-transferrin source of iron binding.

Availability of iron added to TPN CAA as ferrous citrate. This was measured in two ways: (1) Determination of plasma iron and UIBC. Aliquots of the stock ferrous citrate solutions containing 5 mg iron/ml were used to enrich 5 identical TPN CAA formulations to a final concentration of 20 mg iron/liter TPN. A 0.3-ml sample from each of these solutions was then added to 4.5 ml of a freshly-drawn normal human plasma sample of known plasma iron and UIBC. After incubation for 30 min at room temperature, duplicate plasma iron and UIBC measurements were then made, with untreated plasma samples as a control.

Gel filtration. This method was used to separate protein bound iron from unbound iron, the latter either eluting at the bed volume of the column or remaining adsorbed to the gel bed.[16]

A sample of the trace-labeled ferrous citrate solution containing 75 μg iron/ml was added to TPN CAA to a final concentration of 20 μg iron/ml. 0.25 ml of this enriched TPN solution was added to 5.0 ml of a freshly drawn normal plasma sample of known plasma iron and UIBC. The amount of iron present in the added TPN was sufficient to saturate approximately 30% of the plasma UIBC. 1.0 ml of this plasma + TPN mixture was applied to a Sephadex G50 column (50 × 1.6 cm, bed volume = 90 ml, void volume = 36 ml) equilibrated and eluted with normal saline, pH 7.4. Two ml fractions were collected at a flow rate of 30 ml/h and all fractions were monitored for ^{59}Fe activity.

In Vivo Studies

Seven subjects, all maintained on home TPN, gave their informed consent for participation in this study, which was conducted in accordance with the principles for human experimentation summarized in the Declaration of Helsinki. Their clinical profiles are shown in Table I. Each took part in a minimum of two and a maximum of four studies which were conducted on consecutive days at the Clinical Research Center, University Hospital. All the subjects infused TPN CAA from 8 pm to 8 am through a central catheter at a constant rate maintained by an IMED 922 pump. These solutions were identical to those routinely infused by each subject at home, except on those occasions that iron as ferrous citrate was added. Each subject was studied once without any added iron in the TPN CAA infusion. Three subjects were studied with 2.5 mg added iron (subjects 2, 5, and 6), six with 5 mg added iron (subjects 1, 2, 3, 4, 5, and 7) and three with 10 mg added iron (subjects 3, 5, and 7). The iron, which was prepared as described in "Materials and Methods," was added in a volume of 5 ml pH 2 saline to 2 liters of TPN CAA immediately before the start of the infusion. In all of the subjects, the added iron was tagged with 2 to 3 μc of ^{59}Fe on one occasion.

Before each infusion was started and at 3-hr intervals during the infusion, 3 ml of venous blood was taken from a butterfly needle in a forearm vein. Serum iron and total iron binding capacity (TIBC) were determined on these samples by the ICSH method.[13, 14] In those studies where the added iron was tagged with ^{59}Fe, a measured amount of the infusate was kept as a standard and a 5-ml blood sample was taken at 12 days for determining red cell incorporation of radioiron. In estimating utilization, blood volume was calculated from the height and weight of the subjects.[15]

RESULTS

The mean iron content of five batches of TPN CAA was 0.043 (±0.03) mg/l. By comparison, the mean iron content of four batches of TPN PH was 1.11 (±0.06) mg/l. Separate examination of six batches of the 10% Travamin solution, which is diluted 50% with hypertonic dextrose in formulating TPN PH, showed an iron content of 2.4 (±0.24) mg/l. No measurable iron was found in the other constituents of TPN PH.

Inasmuch as most patients receive 2 liters of TPN solution a day, the iron supply from TPN CAA and TPN PH would be 0.08 and 2.2 mg, respectively.

The availability of the iron already present in TPN PH was examined by incubating 4 ml of the solution, having an iron content of 4.8 μg, with 2 ml of normal plasma having an unsaturated iron binding capacity of 4.4 μg. Despite the fact that there was sufficient iron in the TPN solution to more than saturate the UIBC of the plasma, only 0.6 μg or one-eighth of the TPN PH iron was bound by transferrin.

These results differed from those obtained with TPN CAA containing added ferrous citrate. When aliquots of

TABLE I
Clinical profiles of TPN patients participating in the study

Subject no.	Age/Sex	Wt	Underlying disorder	Duration of TPN therapy	Fe/TIBC/%Sat (μgm/100 ml)	Ferritin (ng/ml)	Hematocrit
		kg			*μg/100 ml*	*ng/ml*	
1	60 M	55.4	Amyloidosis	1 month	79/321/25	292	31
2	56 F	52.0	Crohn's disease	2 months	69/364/19		35
3	23 F	60.1	Short-bowel syndrome	2 years, 8 months	98/520/19	102	37
4	26 M	48.1	Crohn's disease	1 month	64/438/14	54	40
5	59 F	45.4	Pseudo-obstruction	2 years, 8 months	46/413/11	39	
6	61 F	48.0	Crohn's disease	3 years	88/289/30	347	33
7	25 M	63.6	Crohn's disease	1 month	30/351/9	65	42

this enriched TPN CAA solution were incubated with plasma, 75.8% (S.D. ± 1.9%) of the iron was available to transferrin. Furthermore, this availability did not change during storage of the ferrous citrate solutions for 9 months.

The availability of the added iron was further examined by gel chromatography. As shown in Figure 1, 74% of the radioactivity added to TPN CAA as ferrous citrate was found in the transferrin peak after filtration through the Sephadex G 50 column. Thirteen percent eluted at the bed volume and the remaining 13% remained adsorbed to the gel bed, as would be expected of polymerized iron.[16]

In evaluating possible toxicity of the iron-fortified solution, it was necessary to determine whether the injected iron exceeded the binding capacity of the subjects' plasma transferrin. Results of infusing 2 liters of TPN CAA enriched with varying doses of iron up to 10 mg are shown for seven subjects in Figure 2. No untoward subjective effects were noted and in only one subject, receiving 5 mg of iron, was saturation approached. Yet this iron was well utilized, with a mean 81% (range 69–97%) appearing in circulating red cells at day 10.

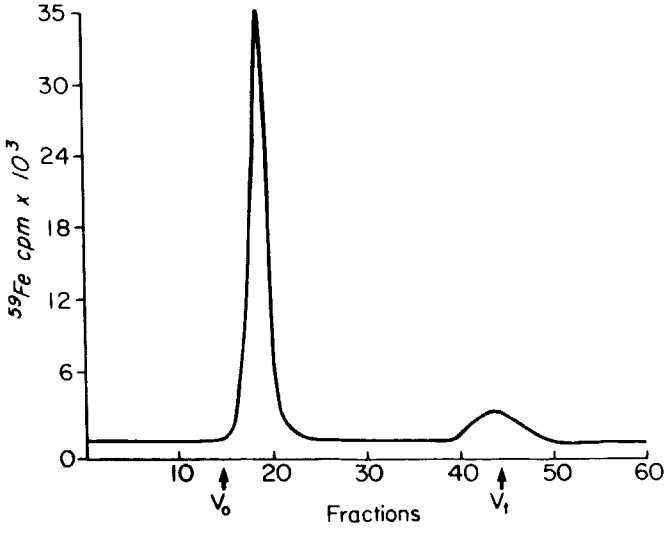

FIG. 1. Sephadex G 50 elution profile of ^{59}Fe ferrous citrate supplemented TPN CAA incubated with normal plasma (cpm = counts/min, V_o = void volume, V_t = bed volume).

DISCUSSION

A number of aspects of iron balance in patients on TPN deserves special consideration. The average iron requirements of nonmenstruating adults are about 14 μg/kg/day, or, considering the weight of the patients on TPN, about 0.75 mg/day. Iron loss from the gastrointestinal tract, either as exfoliated cells or extravasated blood, accounts for 60% of this physiological loss in nonmenstruating adults. In TPN patients such predictions may be inappropriate. On the one hand, the contribution of the gastrointestinal tract to iron loss may be negligible in patients who have undergone extensive resections, whereas, on the other hand, patients with active inflammatory bowel disease are at greater risk of iron deficiency by virtue of increased blood loss. Other considerations include the use of aspirin for analgesia and the iron lost in frequent blood sampling for laboratory investigations.

In contrast to the normal individual whose intestinal iron absorption is regulated, these individuals are completely dependent on the amount of iron provided by TPN. The iron content of the TPN formulation containing Travamin (TPN PH) would appear more than ample, exceeding daily requirements. The limited immediate availability of such iron to transferrin should not imply that this iron is unavailable within the body. However, it has been pointed out that batch-to-batch variation in the amount of iron present is such that a fixed dose cannot be predicted.[17]

At the time our studies were being conducted, the team supervising the home TPN program decided to substitute Travasol for Travamin in the formulations infused by all the TPN patients. This was in recognition of the fact that nitrogen utilization is better from the crystalline amino acid preparation.[18] This policy enhanced the relevance of our studies considerably, especially as Travasol contains very little iron. Our results showed that an infusion of a 2-liter solution, containing 1 liter of Travasol, provided less than 0.1 mg of iron. This is about one-seventh of the daily requirement for an

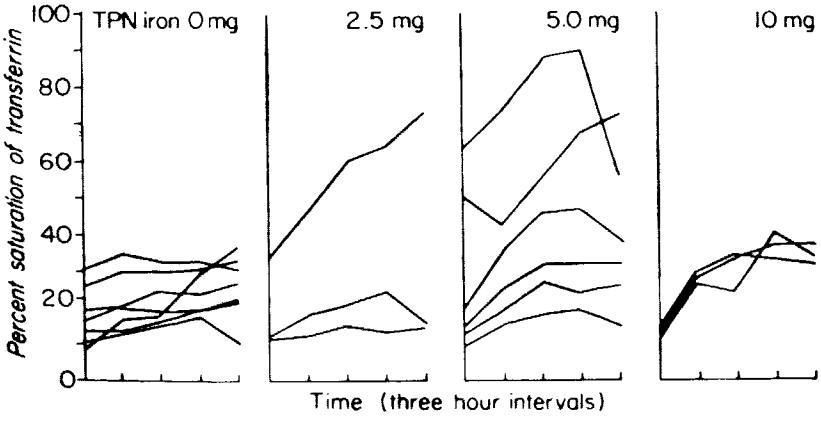

FIG. 2. Changes in percentage saturation of circulating transferrin at 3-hr intervals (8 pm–8 am) during infusion of TPN CAA supplemented with different amounts of iron as ferrous citrate. (The subjects taking part, listed from highest to lowest starting percentage saturation are as follows: 0 mg, numbers 6, 1, 2, 3, 4, 5, 7; 2.5 mg, numbers 6, 2, 5; 5.0 mg, numbers 3, 7, 2, 1, 4, 5; 10 mg, numbers 3, 7, 5.)

average adult and an even smaller fraction of the requirement for adults with superimposed blood loss due to menstruation, blood sampling or gastrointestinal bleeding.

The decision to add an iron supplement to the infusions of patients exclusively dependent on TPN is best made by considering serial measurements of hematocrit, red cell indices, plasma iron concentration, total iron binding capacity, and plasma ferritin concentration. It has been our experience, however, that the assessment of iron nutrition in many TPN patients is complex and it is often difficult, short of staining a marrow aspirate for iron, to decide if a reduced hematocrit and mean cell volume is due to the relative iron deficiency of chronic inflammation or absolute iron deficiency. In the former, the features of iron-deficient erythropoiesis are present because of a block in the release of iron from the reticuloendothelial system and, provided iron stores are present, iron supplementation would not be expected to repair the hematocrit. By comparison, patients with absolute iron deficiency would benefit from supplementation. The measurement of iron stores is then particularly important in establishing a patient's candidacy for added iron. In this context, we place considerable importance on measurement of the ferritin and the fact that its concentration is proportional to the size of the body iron stores. In the uncomplicated patient, we regard a concentration less than 12 ng/ml as indicative of iron depletion.[19] In the presence of inflammation, although a relationship between the plasma ferritin and iron stores persists, it is at a higher concentration of ferritin. For this reason, the corresponding plasma ferritin concentration identifying iron depletion in the patient with inflammatory disease is 50 ng/ml.[20]

Although we currently achieve iron balance in these patients by giving iron dextran, either as a regular additive to their TPN solutions or as less frequent total dose infusions, we recognize the drawbacks to this approach. In a recent study, 53 of 481 patients, or 11%, experienced systemic reactions to intravenous iron dextran.[10] This is an unacceptable hazard for the unsupervised TPN patients infusing solutions at home. Intravenous iron dextran also confuses the interpretation of plasma iron and iron-binding capacity measurements. This is because the compound, which is not distinguished as nontransferrin iron in the routine methods for measuring plasma iron, may circulate for many days. Under these circumstances, an iron-deficient individual may have a spuriously elevated plasma iron. The availability of iron dextran is also limited. The average utilization in iron deficient patients has been measured at 39% after 3 weeks.[21] By comparison, the iron citrate solution used to fortify TPN CAA in our studies was shown to be rapidly available to transferrin *in vitro*. What is more, this form of added iron is well utilized in hemoglobin synthesis with a mean incorporation of 81% at 10 days. When given in doses which exceed daily requirements, the iron citrate was well tolerated and no reactions were observed. This experience has prompted a study which will examine the long-term efficacy of ferrous citrate as an additive in TPN solutions.

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