Pro-oxidant Effects of Iron in the Newborn Period

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Iron, a transition metal, can take part in redox processes by undergoing reversible valency changes. It plays an essential role in oxygen transport by hemoglobin in erythrocytes, oxygen storage by myoglobin in muscle, and electron transfer and energy metabolism in mitochondria. It is also a cofactor in various enzymes. Iron deficiency can produce adverse effects because of anemia in all age groups, but in the child it may also impair neurodevelopment. Worldwide attempts are made to prevent iron deficiency, and iron supplementation is recommended for at-risk groups (1).

Recently, concern has arisen that universal supplementation may also sometimes be harmful (1,2). Excess iron supply has been linked to heart disease and malignancy in older patients (1,3).

These problems do not appear to have direct implications for the pediatrician. Iron deficiency is the main problem in children, especially when they are growing rapidly. However, iron overload may occur more frequently in neonatal nurseries than we generally realize. Although inherited neonatal hemochromatosis is a rare disease, other causes of iron overload may be more common. One example may occur in preterm babies. Because of their poor endogenous reserves and rapid postnatal growth, it is recommended that such infants begin iron supplementation from the eighth postnatal week. However, if they are fed on preterm formula, usually iron fortified, their iron supplementation starts much sooner. This early dietary supplementation may be harmful to some of the babies. Another example is often encountered in our perinatal unit, the center for rhesus hemolytic disease in The Netherlands. Babies with hemolysis and raised ferritin levels nevertheless receive dietary iron if fed on formula feeds (4). Specific contraindications to iron supplementation may need to be agreed upon and the timing of the start of supplementation more stringently controlled (2).

In this chapter we review the role of iron in the pathogenesis of free radical damage in the newborn baby. The available evidence on how nutrition may influence the pro-oxidant activity of iron in the baby is discussed.
ROLE OF REACTIVE OXYGEN SPECIES IN THE PATHOGENESIS OF DISEASE IN THE NEWBORN

Reactive oxygen species (ROS), by damaging proteins, lipids, and DNA, can play a role in the pathogenesis of diseases such as hypoxic/ischemic encephalopathy, intraventricular hemorrhage, retinopathy of prematurity, chronic lung disease, and necrotizing enterocolitis. Preliminary work also implicates ROS in parenteral nutrition induced cholestasis and rhesus hemolytic disease (5). Recent research suggests that the redox status of cells can also influence disease processes by influencing membrane receptors, enzyme activity, signal transduction and transcription, and gene expression (6). Antioxidant defenses, made up of intracellular and extracellular components, work synergistically to prevent oxidative damage and help maintain the optimal redox balance of tissues for normal metabolic activity, growth, and development.

The ROS include inorganic and organic oxygen-derived compounds, some of which are free radicals (one or more unpaired electrons in their atomic orbits)—that is, superoxide radical (\(-\text{O}_2^\cdot\)), hydrogen peroxide (\(\text{H}_2\text{O}_2\)), hydroxyl radical (\(-\text{OH}\)), hypochlorous acid (\(\text{HOCl}\)), nitric oxide radical (\(\text{NO}^\cdot\)), and alkoxyl and peroxyl radicals (\(\text{RO}^\cdot\) and \(\text{ROO}^\cdot\)). \(\text{NO}^\cdot\) reacts rapidly with \(\text{O}_2^\cdot\) to form the strong oxidant peroxynitrite (\(\text{ONOO}^-\)) (7,8).

The ROS pool depends on the balance between their input (exogenous and endogenous sources) and their output (rate of removal), and a sink model is useful in analyzing the processes that can influence this pool (Fig. 1) (5).

**Input**

Although the diatomic oxygen molecule (\(\text{O}_2\)) is itself a radical with two unpaired electrons, its reaction with biomolecules is spin restricted: the parallel spin of the two electrons restricts its ability to oxidize other nonradical molecules containing atoms that are usually bound covalently by electrons in opposite spin (7). However, \(\text{O}_2\) can be converted to highly active ROS. During energy production in the mitochondria most of the \(\text{O}_2\) undergoes a four-electron reduction to water, but a normal small, accidental leak of electrons does occur, producing a single-electron reduction of \(\text{O}_2\) to \(\text{O}_2^\cdot\). Eicosanoid metabolism also produces \(\text{O}_2^\cdot\). Much larger and potentially damaging amounts of \(\text{O}_2^\cdot\) are released when mitochondria are damaged or when xanthine oxidase in various cells or NADPH oxidase in neutrophils is activated by ischemia/reperfusion injury. The \(\text{O}_2^\cdot\) can be further reduced to \(\text{H}_2\text{O}_2\), either spontaneously or through superoxide dismutase activity. If it is not catabolized the \(\text{H}_2\text{O}_2\) will, in the presence of nonprotein-bound transition metals in the reduced form (e.g., ferrous [\(\text{Fe}^{2+}\)] ions) be converted to \(\text{OH}\), the most reactive oxygen metabolite. Nonprotein-bound iron (NPBI) is normally present in only minute amounts in cells and is not present in plasma. Ceruloplasmin oxidizes iron, which is then rigorously bound to transferrin in plasma, whereas ferritin—the intracellular iron binding protein—has an intrinsic ferroxidase activity (7). These proteins are referred to as preventive antioxidants, since they inhibit \(\text{OH}\) production.
FIG. 1. Simplified sink model showing how an imbalance in the input or output of reactive oxygen species (ROS) may result in an overflow, causing molecular damage or alterations in redox-dependent essential cell activities (e.g., signal transduction). The production of ROS (e.g., by mitochondria, xanthine oxidase, and neutrophils) contributes to the input of ROS. The preventive antioxidant capacity of ceruloplasmin and transferrin, which, respectively, oxidize and bind iron and thus inhibit conversion of \( \text{H}_2\text{O}_2 \) to \( \text{OH}^- \), are emphasized. The role of antioxidant enzymes and chain-breaking antioxidant substances (e.g., vitamin E) in controlling the output of ROS is shown. Note that uric acid and bilirubin can act as preventive and chain-breaking antioxidants, whereas vitamin C may have pro-oxidant as well as antioxidant activity.

Another important ROS is \( \cdot\text{NO} \), which is produced by endothelial cells, neurons, and activated macrophages and neutrophils. \( \cdot\text{NO} \) can react with \( \cdot\text{O}_2^- \) to produce the powerful oxidant ONOO\(^-\).

**Output**

The output of ROS depends on antioxidant enzymes and antioxidant substances. \( \cdot\text{O}_2^- \) is converted by superoxide dismutase to \( \text{H}_2\text{O}_2 \), which is catabolized by catalase, glutathione peroxidase, or both. The synergistic action of chain-breaking antioxidants (e.g., vitamins E and C, uric acid, bilirubin, sulphydryl groups, and various “unidentified” antioxidants) also prevents oxidative damage (5). These antioxidants sacrifice themselves by forming stable radicals, thus inhibiting propagation of the oxidative process—that is, continuation of a chain of radical reactions between more reactive unsaturated lipids and proteins (see later).
Imbalance Between Input and Output

An increased production and/or decreased removal of ROS—that is, an imbalance between input and output results in accumulation of ROS. Intracellular antioxidant enzymes form the major component of the antioxidant system; however, immaturity may increase the relative importance of extracellular antioxidants (5). In this review we will concentrate on the role of plasma antioxidants in preventing iron-induced oxidative damage in the preterm and term newborn infant.

THE MECHANISM OF IRON-INDUCED OXIDATIVE DAMAGE

Transition metals (e.g., iron and copper) are found at the active sites of oxidase and oxygenase enzymes because they can accept and donate single electrons and thus overcome the spin restriction of O₂. However, this ability can cause harm if the iron is “free”: NPBI in the reduced ferrous form (Fe²⁺) can reduce H₂O₂ to ·OH (Fenton reaction): Fe²⁺ + H₂O₂ → Fe³⁺ + ·OH + OH⁻. The Fe²⁺ can also react with (i) lipids, (ii) proteins, and (iii) DNA.

Reaction with Lipids

Fe²⁺ reacts with lipid peroxides (ROOH) (and endoperoxides produced by cyclooxygenase) to form damaging RO· and ROO· radicals that can extract H from polyunsaturated fatty acids (RH) and thus initiate a chain reaction of peroxidation:

\[
\text{Fe}^{2+} + \text{ROOH} \rightarrow \text{Fe}^{3+} + \text{ROO}· + \text{OH}^-
\]

\[
\text{ROO}· + \text{RH} \rightarrow \text{ROOH} + \text{R}·
\]

\[
\text{R}· + \text{O}_2 \rightarrow \text{ROO}·
\]

Reaction with Proteins

Although initially most attention was paid to the effects of iron-induced damage on lipids, it is clear that iron-induced oxidative damage of amino acids (e.g., histidine, tyrosine, phenylalanine, or cysteine) can also have major repercussions such as inactivation of glutamine synthetase in the brain or α-1-proteinase inhibitor in plasma (8).

Reaction with DNA

Iron-induced DNA damage can occur: ·OH radical can oxidize the DNA bases to produce compounds such as 8-oxoguanine and 2-hydroxyadenine, as well as causing strand scission. Low-molecular-weight iron can passively diffuse from the cytoplasm across the nuclear pores, but there is also an active nuclear iron transport system. The
normal function of nuclear iron has not been established, but iron overload could damage DNA by the Fenton reaction (3).

PROTECTION AGAINST IRON INDUCED OXIDATIVE DAMAGE

Production of OH, through the catalytic action of NPBI, must be prevented. Thus iron is predominantly incorporated into proteins (e.g., enzymes) and is rigorously bound when transported by transferrin in plasma or lactoferrin in milk, or when it is stored in ferritin or hemosiderin. Normally, NPBI is absent in extracellular fluids and is only present in very small concentrations in cells—as chelates of citrate, ATP, or ADP—during transfer from transferrin or ferritin into hemoglobin or enzymes. The iron content of plasma normally depends on the balance between input (from exogenous supply through absorption from the intestine and release from the endogenous storage depots ferritin and hemosiderin) and endogenous output into tissues such as bone marrow. Except during menstruation, little iron is normally lost exogenously (through skin and intestinal mucosal cell turnover).

The iron must be in the oxidized Fe$_{3+}$ form before it can be bound by transferrin, lactoferrin, and ferritin. The Fe$_{2+}$ is oxidized in plasma to Fe$_{3+}$ by the ferroxidase activity of ceruloplasmin, but ferritin has an intrinsic ferroxidase activity. This oxidizing activity can be antagonized by reducing agents, in particular high concentrations of vitamin C (Figs. 1 and 2A). It has been suggested that megadoses of vitamin C taken by iron-repleted adults for antioxidant protection might therefore act as a pro-oxidant and initiate peroxidative damage (9). Transferrin and lactoferrin bind iron more rigorously than ferritin. Ferritin iron, unlike iron in transferrin and lactoferrin, is released in the presence of reducing substances such as -O$_2$ and vitamin C. However, transferrin, unlike lactoferrin, will release its iron in an acidic environment (pH <5.5). There is evidence that uric acid and bilirubin, as well as being powerful chain-breaking antioxidants, may also bind iron (Fig. 1) (5,10). This could offer some additional protection against iron-induced oxidative damage in the newborn, but the levels of these two substances often fall rapidly postnatally owing to renal immaturity and phototherapy, respectively (5).

Clinical studies to investigate iron-induced stress have concentrated on extracellular measurements. Gutteridge has developed methods to assess the iron-oxidizing (ceruloplasmin) and iron-binding (transferrin) antioxidant capacity of plasma, as well as to test for the presence of NPBI (Fig. 2A and 2B; Fig. 3) (7). The antioxidant capacities of ceruloplasmin and transferrin are measured in vitro as their ability to inhibit peroxidation of unilamellar liposomes containing unsaturated lipids. By altering the concentrations of either iron or vitamin C in the test system, the capacity of either ceruloplasmin or transferrin in a patient’s plasma can be tested (Figs. 2A and 2B). Various methods are available to measure NPBI (“free iron”) (7,11); the commonly used bleomycin test is illustrated in Fig. 3 (7). The test is based on the well-described pharmacological action of bleomycin, an antitumor antibiotic, that initiates strand breaks in DNA by oxidative damage. It binds to DNA and then chelates NPBI, which, if then reduced by vitamin C, produces oxidative DNA damage. The breakdown products react with
FIG. 2. *In vitro* tests to assess the iron-oxidizing and iron-binding antioxidant capacities of, respectively, plasma ceruloplasmin and transferrin. The percent inhibition of peroxidation of polyunsaturated fatty acids, in liposomes, by plasma is assessed (i.e., the fall in malondialdehyde production as measured by the thiobarbituric acid [TBA] reaction). (A) Ceruloplasmin ferroxidase activity. Plasma is incubated in the presence of liposomes and a high concentration of iron. The excess of added Fe$^{3+}$ will occupy all available transferrin binding sites in adults as well as babies and free (non-protein-bound [NPBI]) iron will always occur. Pro-oxidant Fe$^{2+}$ will form if the ferroxidase capacity of ceruloplasmin is limited despite the low vitamin C concentration. Plasma with low ceruloplasmin levels (e.g., in preterm babies) can only partly inhibit oxidative damage of liposomes compared with plasma of adults. (B) Transferrin iron-binding capacity. Plasma is incubated in the presence of liposomes and a high concentration of vitamin C. The free iron-binding sites on transferrin bind the trace amounts of intrinsic iron (from glassware). The excess of added vitamin C antagonizes the ferroxidase activity of ceruloplasmin, and any free iron will be reduced to Fe$^{2+}$ and catalyze lipid peroxidation. Plasma with low concentrations of transferrin and/or highly iron-saturated transferrin (e.g., from preterm babies) can only partly inhibit lipid peroxidation compared with plasma of adults.
FIG. 3. The bleomycin assay for non-protein-bound iron in extracellular fluids (e.g., plasma). Bleomycin requires the presence of iron to degrade DNA. Bleomycin does not remove iron from proteins, and a positive test indicates the presence of non-protein-bound iron (NPBI, free iron). Bleomycin attaches to DNA and binds NPBI, which if reduced by added vitamin C will degrade DNA to malondialdehyde, which can be measured spectrophotometrically as a pink thiobarbituric acid adduct.

thiobarbituric acid to form a pink chromagen that can be measured. Thus the presence of NPBI in extracellular fluid can be measured by the degree of DNA damage using this in vitro test. Gutteridge et al. have confirmed the biological relevance of the test: when the NPBI test is positive in babies' plasma, the “free iron” present is also available for use as a cofactor by aconitase (12). These tests have been used to compare normal and iron-overloaded children and adults (e.g., in kwashiorkor, thalassemia, and hereditary hemochromatosis) as well as in healthy and ill newborn babies.

EVIDENCE OF LIMITED PROTECTION AGAINST IRON-INDUCED OXIDATIVE DAMAGE IN THE NEWBORN BABY

Plasma NPBI is only detected in older patients when they are ill and have excess iron or decreased iron-binding capacity. However, plasma NPBI is commonly present in cord blood, even in well babies (13,14). This is probably related to their relatively low transferrin and ceruloplasmin concentrations as well as to the very active transplacental iron and vitamin C transport (15). The possible role of this NPBI in the pathogenesis of various diseases in babies is increasingly attracting attention.

Scott et al. first drew attention to the fact that transferrin was highly loaded with iron in cord blood plasma (16). Because certain pathogenic microorganisms are iron dependent, this group speculated that early iron supplementation could increase the risk of neonatal sepsis. More recently, it has been realized that iron overload may also cause damage by initiating oxidative processes, and Sullivan was the first to suggest that this process could be important in the newborn infant (17). He hypothesized that iron-induced oxidative damage could lead to retinopathy of prematurity, intraventricular hemorrhage, bronchopulmonary dysplasia, and necrotizing enterocolitis. Despite the steadily increasing number of in vitro and in vivo studies, the role of iron-induced oxidative damage in neonatal diseases and its interaction with nutrition and other treatments in the neonatal period is still poorly understood.
Cord blood plasma transferrin concentrations correlate positively with gestational age, but even at term they are much lower than in adults (16). The levels in growth-retarded babies are similar to those in well-nourished newborn babies. In the baby, transferrin is much more highly loaded with iron than in later life. The calculated transferrin saturation is around 60% in babies, compared with 40% in adults (16); however, in a recent study where babies’ transferrin saturation was measured, it was found to be even higher, with levels of 100% being common (18). The formula to calculate transferrin saturation uses the molecular mass of transferrin and may not be appropriate for babies. Transferrin is not a homogeneous substance and variations in the polypeptide and glycan chains contribute to its heterogeneity. Different subtypes occur in the preterm and term newborn baby (19), and this could affect the iron-binding capacity. Recently, NPBI has been detected in the plasma of healthy preterm and term babies (13,14). The need to build up adequate iron reserves in the last trimester may be the teleological explanation for the highly iron-loaded transferrin, and this may carry little risk in the low-oxygen environment in utero. However, after birth the situation changes dramatically and the presence of NPBI may be dangerous in the high-oxygen environment of terrestrial life.

Recent studies show that both the preterm and the term baby have diminished ability in vitro to inhibit lipid peroxidation owing to the limited iron-binding capacity of their transferrin and limited iron-oxidizing capacity of their ceruloplasmin (Figs. 1 and 2) (15). The decreased iron-binding capacity is related to their low transferrin concentrations as well as to the high iron saturation. The role of qualitative factors (e.g., amino acids, glycans, and bicarbonate ion in transferrin) is unknown. The decreased iron-oxidizing capacity is due not only to their low plasma ceruloplasmin levels but also to the high plasma vitamin C level at birth, which antagonizes the ferroxidase activity of ceruloplasmin (Figs. 1 and 2A) (15). The vitamin C concentration falls rapidly and is much lower by the third day in even fairly mature preterm babies (5). There is a negative correlation between ferroxidase activity and the vitamin C/ceruloplasmin ratio in babies (11). Thus any NPBI would then theoretically exist in the pro-oxidant Fe^{2+} form, and Berger et al. have recently shown that the NPBI was indeed present in cord blood plasma as Fe^{2+} (20). Little is known about how the postnatal changes in iron and vitamin C levels and transferrin and ceruloplasmin activities may affect the presence and toxicity of NPBI. An important clinical study has shown that babies with NPBI even in the presence of high vitamin C levels do not have increased concentrations of F_{2}-isoprostane and carbonyl, oxidative products of lipids and proteins, respectively, in their cord blood plasma (21). On the basis of further in vitro studies the authors suggested that, although vitamin C can act as a double-edged sword and have either pro-oxidant or antioxidant effects, the very high levels in the newborn, even in the presence of NPBI, have a net antioxidant effect (21). However, we have shown that vitamin C levels fall dramatically postnatally (5). Thus we suggest that the situation might change to a pro-oxidant state postnatally if NPBI persists. We are in the midst of analyzing the serial postnatal changes in NPBI in babies who participated in a feeding trial. Preliminary results suggest that NPBI levels in plasma do fall rapidly postnatally (22).
THE CLINICAL EVIDENCE THAT IRON PLAYS A ROLE AS A PRO-OXIDANT IN NEONATAL DISEASE

There is now preliminary evidence incriminating iron-induced oxidative damage in the pathogenesis of rhesus hemolytic disease (4), neonatal respiratory distress syndrome (RDS) (14), bronchopulmonary dysplasia (23), hypoxic/ischemic encephalopathy (24), and retinopathy of prematurity (25). There appear to be no clinical studies relating iron to the development of necrotizing enterocolitis, but iron does play an important role in animal models of this disease (26). There is a clear link between nutrition and necrotizing enterocolitis (27), and the potential interaction in the intestinal lumen of iron, vitamin C, and long-chain polyunsaturated fats requires clinical and experimental investigation.

In rhesus hemolytic disease, excess iron release from hemolysis may overload the transferrin and damage the liver and endothelial cells, producing hydrops fetalis by oxidative mechanisms (4) analogous to those occurring in thalassemia, and hemochromatosis in older patients (28). Babies with rhesus hemolytic disease have raised ferritin levels, a lowered latent iron-binding capacity, increased lipid peroxidation products, and decreased vitamin C concentrations in their plasma.

In RDS, leakage of plasma containing NPBI into the alveolar space could aggravate surfactant losses by inducing peroxidation (14). The ability of plasma from preterm babies to inhibit iron-catalyzed lipid peroxidation of pulmonary surfactant in vitro was lower than that of term babies and was related to the presence of NPBI. Babies with RDS who subsequently develop chronic lung disease have lower transferrin levels than those who recover uneventfully from RDS. The fall in transferrin (a smaller molecule than ceruloplasmin) may be due to leakage into the alveoli (23) caused by oxidative damage, and this could aggravate oxidative damage in the alveolar space (11,14).

In hypoxic/ischemic encephalopathy the energy depletion, acidosis, and superoxide production may release iron from tissue stores in the brain and liver (24). Plasma NPBI is present in asphyxiated babies; this may not only reflect the unbound iron status in the brain but also directly contribute to ischemic reperfusion injury (24). Extracellular oxidative events may, for example, damage endothelial cell membranes, aggravate the cerebral edema, and adversely contribute to the long-term outcome of the babies.

Thus even brief temporary disturbances in iron binding could have serious long-term effects on the growth and development of many tissues in the baby.

THE POSSIBLE INTERACTION OF VARIOUS TREATMENTS AND/OR DIET ON IRON-INDUCED OXIDATIVE DAMAGE IN THE NEWBORN

At present, treatment of ill or preterm babies probably inadvertently inhibits or aggravates iron-induced oxidative stress, but in future, specific prevention of this problem may be possible (4) for a number of important neonatal (and pediatric) diseases. Possible therapeutic steps could include lowering iron and raising vitamin C concen-
trations, or iron-binding and iron-oxidizing activity of the plasma. The iron content of plasma is controlled by input from exogenous and endogenous sources, but the only significant output is through cell uptake, as exogenous losses are minimal. The large number of transfusions that preterm babies receive can more than compensate for their missed transplacental supplies. Furthermore, intrauterine transfusions in rhesus hemolytic disease may cause iron overload, and babies with this disease should not receive preterm formulas containing extra iron in early life (4). It appears that, unlike in older patients, iron overload from blood transfusions does not result in downregulation of intestinal iron absorption in preterm infants (29). Therefore early feeding of iron-supplemented formulas to babies could aggravate any iron overload in those requiring transfusions. Blood transfusions in babies with bronchopulmonary dysplasia did increase the incidence of NPBI, although there was no increase in lipid peroxidation products in the plasma (30). Formulas with a high iron, low vitamin E, and high polyunsaturated fatty acid content can cause hemolytic anemia (31). We compared the effect of early iron supplementation through preterm formulas on NPBI levels and oxidative stress in preterm infants (gestational age 27 to 34 weeks) fed an iron-fortified preterm formula (0.8 mg/dl iron) with a group of infants fed the same formula without added iron (0.08 mg/dl iron). Formulas were given as soon as the infants tolerated enteral feeds. Iron supplementation did not result in a different incidence of NPBI, or different levels of lipid peroxidation products and antioxidants such as vitamins C and E. However, ferritin concentrations were lower in the non-supplemented group, and 17% of these infants had deficient values at the end of the study period, compared with none in the supplemented group (22). Parenteral iron may increase oxidative stress when used in all-in-one solutions (32) or when provided with erythropoietin treatment to prevent early anemia in preterm babies. However, when given alone, erythropoietin actually protects the lung of premature rabbits by decreasing the iron loading of transferrin (33).

The iron-oxidizing and iron-binding capacity may also be inadvertently influenced by therapeutic measures. The possible clinical consequences of the antagonistic effect of vitamin C on ferroxidase activity were discussed in the preceding discussion and have been reviewed recently (5). Peroxynitrite, a powerful oxidant product of nitric oxide, releases copper from ceruloplasmin (34). This could increase the risk of free plasma Fe$^{2+}$ in babies treated with nitric oxide for pulmonary hypertension. Antenatal corticosteroids appear to increase ceruloplasmin concentrations, whereas transfusions and exchange transfusions using fresh-frozen plasma may increase both ceruloplasmin and transferrin concentrations and activity (5). Because of the possible risks of viral infection, we are wary of using fresh-frozen plasma unless it is specifically indicated (e.g., in bleeding diathesis or exchange transfusions). However, in future the use of recombinant transferrin proteins may offer the advantages of increased iron binding without the risks of infection (5,14). As well as quantitative changes in transferrin concentration, qualitative changes in its ability to bind iron could occur. Transferrin binding of iron requires the synergistic action of an anion (bicarbonate); therefore acidosis and its treatment could, respectively, decrease or increase plasma iron binding (24). Transferrin can also bind other metals such as
aluminum. It appears that iron successfully competes against aluminum for the binding sites on transferrin (35); however, these studies have not been carried out with the isoforms of transferrin present in the newborn (see earlier). We speculate that the well-recognized aluminum contamination of feeds and infusions could further limit the iron-binding capacity of transferrin in the neonatal period and perhaps initiate iron-induced peroxidation of the unsaturated lipid intake seen in babies on intravenous fat and the newer preterm formulas.

CONCLUSION

Attention has only recently turned to the possible harmful effects of iron. Overload with this transition metal appears to play a major role in inducing oxidative damage in many diseases in all age groups. Newborn infants have a limited antioxidant capacity and may be more sensitive to iron-induced oxidative damage. There is no clear clinical evidence that postnatal iron supplementation contributes to oxidative damage in the newborn studies, but systematic study of this potential problem has only just begun. Iron toxicity may be one of the factors explaining how illness, therapy, and diet interact to influence morbidity and mortality in the newborn.

SUMMARY

Non-protein-bound iron in the reduced ferrous form can act as a powerful pro-oxidant and damage lipids, proteins, and DNA. In vitro and in vivo evidence suggests that these processes play a role in the pathogenesis of diseases such as hypoxic/ischemic encephalopathy, retinopathy of prematurity, respiratory distress syndrome, and rhesus hemolytic disease in the newborn infant. Immaturity, disease, and nutrition can influence iron and vitamin C concentrations as well as the iron-oxidizing capacity of ceruloplasmin and the iron-binding capacity of transferrin, thereby predisposing the baby to iron-induced oxidative damage. It may, however, be possible by providing optimal nutrition and other therapeutic steps to inhibit iron-induced oxidative damage in the newborn period.

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REFERENCES


DISCUSSION

Prof. Heird: I was struck by the fact that the problem seems to be maximal during the first few days of life. Do you think there is a relation between early nutritional management and levels of transferrin and therefore of iron binding?

Prof. Berger: That’s an important aspect. We have been looking at well babies in particular. We are analyzing our babies with chronic lung disease, but I don’t have the results yet. Even though you are probably giving a minimal amount in these first days, there is buildup in that critical period. People are now suggesting that if we give erythropoietin, we need to give intravenous iron as well. That’s as iron dextran, which is rapidly converted to iron. I am concerned that this new therapeutic measure might have an important pro-oxidant effect. This might also be aggravated in babies who are releasing endogenous iron, such as those with asphyxia and acidosis, where plasma iron may be increased.

Prof. Heird: I was more interested in protein and the effects on synthesis of the iron-binding proteins.

Prof. Berger: The half-life of transferrin is 8 days, and that of ceruloplasmin is twice as much, 14 days as I recall. I don’t know whether there is a fall in transferrin in newborn babies with illness because of undernutrition. We have a paper coming out [1] where we followed transferrin levels, ceruloplasmin, and albumin sequentially in babies with and without chronic lung disease after respiratory distress syndrome. It is well known that babies who have RDS have low protein levels because of lung leakage. We were interested in what would happen in chronic lung disease with continuation of the leaky lung problem, or where we’re feeding them poorly. We found a low albumin and transferrin in these babies, which persisted to day 10. Looking at total protein-to-transferrin ratios, we think this was related to leakage into the lung and increased output rather than to decreased production because of poor nutrition.

Prof. Cooke: Like you, we’ve had some experience in measuring free iron using the bleomycin assay. One of our concerns has been that we’ve been unable to demonstrate that in the presence of free iron there are other indicators of excessive lipid peroxidation. One possibility is that the bleomycin assay may be measuring iron that is actually bound, but not to transferrin. It can be very loosely bound to a number of other proteins and be displaced by the assay itself. Alternatively, maybe we don’t have adequate measures of lipid peroxidation that are useful in the newborn. We’ve measured breath pentane and found that it is not correlated with chronic lung disease, paradoxically; it seems to be correlated mainly with brain injury. The brain obviously is a source of large amounts of lipid, and children with brain injury have very high breath pentane. Lipid infusions in TPN produce huge levels of breath pentane, presumably unrelated to lung damage. The other measures that have been used are things...
like malondialdehyde (MDA), but these are very general and very crude measures of lipid peroxidation. The answer may be to look at something much more localized, perhaps measuring MDA or similar markers in lung fluid. We have started doing that and have shown high levels of MDA and very low levels of antioxidants in lung fluid. I think we need to get closer to the source of the damage. Iron may be important, but we've been looking at the wrong outcome markers.

Prof. Berger: Bleomycin is not an easy test. That's one of the problems. When you speak to people of different parts of the world you find they don't use it. Gutteridge has recently been looking at aconitase activity. Aconitase is an enzyme concerned with citrate metabolism, and it needs iron to be activated. He showed that in babies with free iron present in the plasma you can induce aconitase activity, and this correlates with the bleomycin iron present [2]. So there is some confirmation that the bleomycin test is backed up by newer tests. What we then did in our babies was to actually measure iron-binding capacity and showed there was a very strong correlation between the bleomycin iron and the presence of iron-binding capacity or free iron [3]. So there is confirmation that this iron test is reasonable. What I'm concerned about is the possibility that it is not always true that heme iron and hemoglobin iron do not participate in the test. It may be true in adults, but in the baby there could be interference. I agree we need more sensitive markers. As Dr. Frank pointed out, perhaps we should be looking not only at fat peroxidation products, but also at DNA and protein products in plasma as markers of peroxidation.

Prof. Haschke: Does transferrin receptor help to define iron requirements or iron toxicity?

Prof. Berger: There was a recent abstract [4] looking at transferrin receptor in terms of analyzing the hemolytic process and iron overload in rhesus hemolytic disease, but I'm not aware of anything specifically on preterm infants.

Prof. Haschke: Many people may now be concerned about the ratio of vitamin C to iron. In most formulas the molar ratio is about 10:1. Do you see any disadvantage or even potential dangers if such formulas are given to premature infants, especially when there is danger of iron overload?

Prof. Berger: That question is now beginning to be asked, but we don't have an answer as yet. I know that some research is under way in New Zealand looking at the influence of vitamin C supplementation and iron-induced peroxidation.

Dr. Walker: We have been concerned about the possibility that, as an inappropriate response to luminal stimuli, the NFκB transcription factor excessively regulates the upswing of interleukin-8, which causes an inflammatory response. So your hypothesis is that iron is necessary to release NFκB may not be a very good thing in the immature enterocyte and may create further damage. The absence of iron may be an important factor.

Prof. Berger: On looking up the literature on this subject I came across five articles showing that iron can influence the activation of NFκB, though I agree this was not in the intestine but mainly in cultured liver cells. It is, however, going to be a fascinating prospect to alter redox potential in newborn babies and possibly alter not only NFκB but also apoptosis, which we know goes on for weeks after perinatal asphyxia.

Prof. Nowak: Can ferritin-bound iron be displaced under certain clinical situations, such as acidosis or drugs that we use in the nursery?

Prof. Berger: Transferrin iron is very well bound, whereas ferritin iron is more easily released. But transferrin iron can be released with acidosis; in fact, the basis of
the latent iron-binding capacity tests is to make the plasma very acid so that even the transferrin loses its iron. Ferritin is another story. The work of Koster from Rotterdam suggests that ferritin iron release occurs in ischemia reperfusion damage and heart infarcts [5]. He showed that in the presence of superoxide radicals, ferritin releases its iron. So where you have ischemia reperfusion there will be both an increase of free radical superoxide and a release of iron, which the superoxide then can reduce. The superoxide then also contributes hydrogen peroxide, which the reduced iron will convert to hydroxyl radical. So the release of iron from ferritin, which contains a great excess of iron atoms compared with transferrin, might be very important. We see a dramatic rise in iron release in asphyxiated newborns.

Prof. Ziegler: You mentioned the use of intravenous iron dextran in conjunction with recombinant human erythropoietin. Is anything known about what this does to free iron concentrations in plasma?

Prof. Berger: This was the subject of an editorial in the Journal of Pediatrics recently [6]. Dr. Chessex showed last year in the Journal of Pediatric Gastroenterology and Nutrition [7] that when iron sulfate was present in parenteral fluids, there was ongoing peroxidation in the solution, but with iron dextran there was no increase in peroxide production. So iron dextran seems to be inactive and this fits in with the chemical detail. However, when it is taken up by the reticuloendothelial system, it is rapidly converted to iron and then bound to transferrin. Thus, depending on your input vs. your output, it is theoretically possible that there could be free iron in transit, not yet bound to transferrin.

Dr. Chessex: We found that iron dextran actually had a protective effect against peroxide generation in TPN solutions—the opposite of free iron. We don’t understand why. We tested the effect of dextran alone, and it does not have any antiperoxide activity. So it’s the iron dextran complex that has this activity. We thought it might be a spurious in vitro finding, but we found a fall in urinary peroxide excretion in five pediatric patients receiving intravenous dextran iron after they started treatment. So this seemed to confirm what we were finding in vitro. However, most people now use other sources of complex iron since iron dextran can cause anaphylactic reactions.

Prof. Berger: A parallel could be drawn with desferrioxamine, which also chelates iron which is then not normally active. There was a paper in Lancet a couple of years ago describing patients with thalassemia who were being treated with intravenous desferrioxamine who ended up with severe adult respiratory distress syndrome [8]. It was suggested then that, depending on the site, even these iron-chelating agents could release iron.

Dr. Georgieff: We published some relevant research in the American Journal of Physiology a couple of months ago [9]. This was a study on newborn lambs who were phlebotomized and put into iron balance with saccharated intravenous iron at various doses. These ranged from 0 in the control group through 1 mg/kg-d, 2 mg, 5 mg, and 15 mg. The iron was given as boluses every 3 days, so the highest group received 45 mg/kg every 3 days. The experimental blood loss was equivalent to that found in the newborn intensive care unit, about 2 ml/kg daily. We found that we were able to put the animals back into iron balance at 2 mg/kg-d of IV iron. Assuming that a baby absorbs about 33% from the gut, that would be equivalent to an enteral dose of about 6 mg/kg-d, which is consistent with the dose given to babies receiving erythropoietin. We found no evidence of peroxidative damage until we reached the highest dose of
15 mg/kg-d (45 mg/kg bolus every 3 days). The one animal that received that large amount of iron died after the second dose. At 5 mg/kg-d we saw mild changes that were not really very significant. So although in the lamb the transferrin is saturated at a different percentage than in the human baby, it obviously takes a fair amount of iron to get peroxidative damage.

Prof. Haschke: This interaction between vitamin C and ceruloplasmin seems to be a very sensitive issue. Is there any situation where a high copper intake together with low ceruloplasmin synthesis could be pro-oxidative?

Prof. Berger: It is now believed that copper transport is mainly by albumin, and copper on ceruloplasmin is for ferroxidase activity. Could excess copper play a role in oxidative damage? In vitro, copper is a more powerful pro-oxidant than iron, but its role in the newborn has not been explored. It has been shown that copper may be present in excess in some intravenous fluids, particularly albumin preparations, depending on the maker or the source. So there could be an increased input from this source, perhaps more than we realize. However, the major factor is probably a decreased copper output in some babies. Copper is excreted in the bile and it has been shown that in babies with cholestasis there is increased serum copper. We heard yesterday that there may be peroxidative damage in cholestasis, so it may be that copper is playing a role. We studied copper as a pro-oxidant in preterm babies and showed that babies with low albumin levels had a decreased ability to inhibit copper-induced peroxidation [10]. So it is possible that an excess of copper in the presence of a low albumin could give rise to a pro-oxidant state.

Dr. Georgieff: There are certain conditions that result in babies being born with either low or high ferritin. Low ferritin is often seen in babies with intrauterine growth retardation, probably as a reflection of decreased placental transport. And between 50% and 60% of infants of diabetic mothers are also born with low-ferritin iron. I wonder if there is any information on their susceptibility to oxidative diseases?

Prof. Berger: I don’t know anything about the diabetic group, but we were interested in the intrauterine growth retardation, particularly when people started suggesting that necrotizing enterocolitis has a high incidence in intrauterine growth retardation. I wondered whether this might have something to do with iron. We carried out a study in Cape Town on mothers with preeclampsia who had cesarean sections, looking at the babies’ cord blood levels of iron and non-protein-bound iron. The babies were divided into those with intrauterine growth retardation and those of appropriate weight, but we found no difference in transferrin, ceruloplasmin, or non-protein-bound iron levels.

Prof. Lucas: There is concern about iron intake in adults in relation to ischemic heart disease. Could you comment on our management of preterm infants from an iron point of view? These infants will be sent home with an amount of medicinal iron to be taken daily, and this may be doubled with the iron that they also get in an iron-fortified formula; then within weeks of going home, they may also start receiving iron-fortified weaning feeds. So there are multiple sources of iron.

Prof. Berger: That’s an important question. We have grown up with the idea that iron absorption is regulated and that the amount of absorption depends on iron status. But in the preterm baby, at least in the early weeks of life, that may not be so [11]. The iron absorption regulating mechanism appears not to be well developed, and even when a baby is getting blood transfusions there appears to be undiminished iron absorption from the gut. So it’s certainly possible that we could overload these
babies. I'm concerned about the possible effects of this if the baby becomes ill. To draw a parallel with the adult, one theory about iron and atherosclerosis is that a high iron level is not in itself related to increased atherosclerosis, but if there is excess iron in the tissues at the time of myocardial ischemia, this iron is released and triggers oxidative damage. It is a worrying possibility that the preterm baby who suddenly gets ill may suffer in a similar way from release of iron.

Dr. Georgieff: To give the other side of the coin, we are seeing many children in our clinic with extremely low ferritin and TIBC and with hemoglobins of around 5 g/liter. I don’t know how common this is, but I want to make the point that there is probably a very wide range of iron sufficiency and insufficiency in premature infants after discharge home. The children we see with low iron status tend to have been healthy babies of perhaps 26 or 28 weeks of gestation who did not have lung disease, who did not get transfusions, and who were fed either with a low-iron formula or, more commonly, with their own mother’s milk without iron supplementation. On the other hand, there was the article recently in the *Journal of Pediatrics* [12] documenting ferritins of 500 to 700 ng/ml in babies discharged from neonatal intensive care. So here we have a fairly toxic compound with narrow therapeutic range, and I think we need to pay more attention to iron balance, both in the NICU and at follow-up.

Prof. Berger: This is exactly the point I was trying to make. We need something rather more sensitive than stool gazing.

REFERENCES