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Review

Experimental animal model to study iron overload and iron chelation and review of other such models

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ABSTRACT

The disorders of iron overload due to primary or secondary cause are one of the important human diseases leading to high mortality if untreated. To understand this, an animal model has been extensively studied. The source of iron administered to the mode of iron administration that can mimic the iron overload in humans has been studied. A safe and orally active iron chelator is still needed as many of the existing compounds have different types of complications and toxicity associated. Hence having a simple animal model which can be availed quickly and can be used to study various compounds for its iron chelating activity would likely to have immense utility for pharmacological studies. In this review we have shown how, using a simple procedure, a large number of small iron overloaded animals can be produced easily for various studies.

Contents

1. Review

The most important causes of iron overload are hereditary hemochromatosis, hereditary anemias, acquired anemias and other miscellaneous causes such as chronic liver disease, increased dietary intake, Friedreich ataxia and aceruloplasminemia (Table 1) [1]. Transfusional iron overload can be due to disorders such as sickle cell anemia, thalassemia, hemolytic anemia, myelodysplastic syndromes, aplastic anemia, refractory sideroblastic anemia, myelofibrosis leukemias, myeloproliferative disorders, Diamond–Blackfan anemia and chronic kidney disease [2].

1.1. Pathophysiology of iron overload

The tissue damage occurs when the total iron in the body which is normally 2.5–3 g reaches 7–15 g after 30 to 50 transfusions. The most commonly affected organs in iron overload are the heart, liver and endocrine glands. Cardiomyopathy, cirrhosis of the liver, diabetes due to pancreatic islet cell failure, testicular failure, tanning of the skin and joint and bone pain are observed due to iron deposition in these organs [1]. This also leads to a 3 fold increase in the mortality of chronically transfused patients, the most common cause being cardiomyopathy in 30% of the patients [3]. The age of patients undergoing initiation of

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Table 1
Causes of iron overload.

Causes of iron overload	
Hereditary iron overload/hereditary hemochromatosis	HFE associated (Type 1) Non-HFE associated Transferrin receptor 2 associated (Type 3) Juvenile hemochromatosis (Type 2) [Hemojuvelin associated (Type 2A), Hepcidin associated (Type 2B)] Autosomal dominant hemochromatosis—Ferroportin associated (Type 4) DMT1 associated hemochromatosis Atransferrinemia Aceruloplasminemia
Acquired iron overload	Iron-loading anemias (Refractory anemias with hypercellular erythroid marrow) Chronic liver disease Porphyria cutanea tarda Insulin resistance associated hepatic iron overload African dietary iron overload Medicinal iron ingestion Parenteral iron overload [Transfusional iron overload, Inadvertent iron overload from therapeutic injections]
Perinatal iron overload	Neonatal hemochromatosis Trichohepatoenteric syndrome Cerebrohepatorenal syndrome GRACILE (Fellman) syndrome
Focal sequestration of iron	Idiopathic pulmonary hemosiderosis Renal hemosiderosis Associated with neurologic abnormalities

transfusion therapy differs according to the disease; in thalassemia the age ranges from 6 months to 4 years whereas in sickle cell anemia the average age of initiation of transfusion therapy is 13 years [3]. However, for adults suffering from aplastic anemia and myelodysplasia, it is 40 years and 60 years respectively [4,5].

1.2. Chelators in use and its outcome

Chelating agents help reduce iron levels in the body by promoting the excretion of chelated iron. Deferoxamine is a siderophore (iron-binder) derived from the bacterium *Streptomyces pilosus*. It is usually administered as a slow subcutaneous infusion through a portable pump. It forms a 1:1 hexadentate complex with iron and has a half-life of 20–30 min. The chelated iron is excreted in urine and feces. It readily chelates iron from ferritin and hemosiderin but not from transferrin, cytochromes or hemoglobin. It has proved to be useful in chelating iron from the liver and bone marrow [6]. The first orally active iron chelator, Deferiprone, is a member of a family of hydroxypyridine-4-one (HPO) chelators that requires 3 molecules to fully bind iron, each molecule providing 2 coordination sites (bidentate chelation). Its half-life is approximately 2–3 h and the chelated iron is predominantly excreted in urine [7]. Due to rapid metabolism of deferiprone by glucuronidation in the liver to its inactive metabolite, it requires thrice/day administration. To overcome this, a once daily oral iron chelator, Deferasirox, was developed that binds iron in a 2:1 ratio (tridentate complex). Its half-life is 8 to 16 h which allows once a day dosing and the chelated iron is excreted predominantly in feces. Hence deferasirox is more widely accepted [8]. As iron is an essential element in growth and development of many bacteria/parasites, iron chelators have also been investigated in other disorders like cerebral malaria along with antimalarial drugs [9]. Certain degenerative diseases like Friedreich's ataxia, several types of epilepsy (Vogt Koyanagi Harada's disease) iron deposition occurs in specific parts of neuraxia, hence targeted iron chelators towards these areas of brain could also have medical use.

Complications of iron overload have been steadily falling since the introduction of deferoxamine. For thalassemia major patients in Italy who were started on deferoxamine subcutaneous infusions which became widely available in 1980, death from cardiac disease fell from 5% at 20 years to 1% and the incidence rates of hypogonadism, diabetes and hypothyroidism also fell significantly [10]. Better survival has been demonstrated for patients born in more recent years. In a study by Borgna-Pignatti et al. in 2004, 68% of patients were alive at the age of 35 years, with 67% of the patient's deaths due to heart disease. In a few patients who failed to comply with deferoxamine treatment or started the chelation therapy late in life, a high level of iron [>15 mg iron/g of liver (dry weight)] was present which was associated with a high risk of cardiac disease and early death over a long period [11].

Besides these approved iron chelators, many more are under investigation. Iron chelators such as 2-furildioxime, hexadedntate N,N′-di(2-hydroxy)benzylethylene-diamine-N,N′-diacetic acid (HBED), dexrazoxane, pyridoxal isonicotinoyl hydrazone (PIH), desferrithiocin (DFT), CP502, CP360, GT56-272, FDO, Triapine, MPB0201, etc. have been in the developmental stages [12].

1.3. The need for animal model and literature survey

Besides being iron chelating agents, these iron chelators have many side effects. As deferoxamine is administered subcutaneously, toxicity and compliance are the major disadvantages besides its adverse effects which include local reactions, ophthalmologic, auditory, pulmonary and neurologic complications and infections. Administration of deferiprone three times a day on the other hand causes agranulocytosis, neutropenia, arthralgias and arthritis. It is not licensed for use in the United States. Once a day iron chelator-deferasirox was also reported to cause gastrointestinal disturbances, renal insufficiency and neutropenia [13].

Setting up animal models to study iron loading and chelation has been in practice since a long time. Many animals have been tried with different ways to overload iron which can mimic the natural way of iron overload and its related complications.

The consequences of introducing excessive quantities of iron in various forms into the animals have been repeatedly studied since 1928 [14–23]. Studies showed that the exogenous iron in colloidal complex form had a different manner in which it was distributed by the animal body as compared to the characteristic hemochromatosis disease found in humans [24]. With the advent of an iron-dextran complex, further studies were undertaken as iron-dextran possessed unique properties with regard to pH stability, low toxicity and absorption from muscles [25]. However, iron-dextran given parenterally over 60 weeks deposited in the hepatic, renal and testicular organs of albino rats and rabbits and did not show any notable change towards hemochromatosis from normality. Also noticed was a reduction in the serum iron levels to normal in few weeks after the injections were ceased (Table 2) [24].

Bacon et al. in 1983 suggested that parenteral administration of iron (ferric nitrilotriacetate) to rats produces homogenous deposition of iron throughout the hepatic lobule in both hepatocytes and Kupffer cells whereas iron when supplied in diet in the form of carbonyl iron produced predominant hepatocellular iron deposition in a periportal distribution, a pattern analogous to that seen in human hereditary hemochromatosis [26]. The dietary supplements of carbonyl iron to rats was further studied by Iancu et al. in 1987, Park et al. in 1987 and Houglum et al. in 1990 and showed that it result in iron overload leading to lipid peroxidation, tissue injury and tissue fibrogenesis. However this could be achieved only after prolonged feeding of elemental iron for up to 9 months [27–29] and oral iron produces a different pattern of iron deposition compared to transfusional (parental) iron overload [26].

Carthew et al. in 1993 wanted to develop a rodent model with prolonged iron overload which had both cardiotoxic and hepatotoxic effect. After subcutaneous administration of iron dextran to Mongolian gerbils on a weekly basis for 7 weeks, severe hemosiderosis of the liver and heart were seen. This gradually developed into hemochromatosis