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COMMENTARY

Therapy by Taking Away: The Case of Iron

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ABSTRACT. The recent finding of the beneficial effects of iron deprivation in the outcome of muscle necrosis in an animal model of genetic myopathy served as the basis of this commentary. Here, "taking away" iron by controlled dietary deprivation is proposed as a reasonable, feasible, cheap, and efficient clinical approach to many diverse diseases, all of which have a free radical component. Indeed, iron potentiates the generation of the highly reactive and toxic hydroxyl radical, and, thus, of oxidative damage. Iron deprivation may represent the first really efficient antioxidant, preventing oxidative stress in all subcellular compartments, tissues, and organs. Iron/iron deprivation also modulates programmed cell death (apoptosis), which should be the subject of further studies to better define the mechanisms mediating these complex effects. Finally, related to its antioxidant effects, iron deprivation may find applications in the anti-aging field, whether programmed or premature aging, and whether in cosmetics or in gerontology. BIOCHEM PHARMACOL **57**;12:1345–1349, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. reactive oxygen species; iron; iron chelators; Fenton reaction; ischemia-reperfusion; infectious diseases; aging

Classical pharmacotherapy usually ends by giving the patient some magic in the form of a lively, colored, modernly shaped, and shiny pill: something was missing, something good is being administered. Here we propose that many diseases as diverse as myocardial infarction or stroke, inflammatory conditions such as bleomycin-induced pulmonary fibrosis, bacterial infections and malaria, or DMD†, all of which have a free radical component, would actually benefit most from taking away—in this case, iron. Iron deprivation appears to be more efficient and more feasible than any classical antioxidant therapy. Indeed, the latter has been limited by the heterogeneity of the clinical courses of the many diseases in which oxidative stress plays a role, and by homeostatic mechanisms that complicate the specificity of tissue and subcellular targeting of antioxidants. Thus, alternative approaches such as dietary deprivation or iron chelation are being considered as attractive therapeutic avenues, more likely to provide both efficient and safe antioxidant protection than any scavengers, vitamins, or trace elements.

ROS, IRON, AND FERRITIN

ROS include the free radicals superoxide ($O_2^{\bullet -}$) and hydroxyl ($^{\bullet}OH$), the non-radical intermediates singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2), as well as nitric oxide ($^{\bullet}NO$) and peroxynitrites ($ONOO^-$). At low concentrations, ROS can act as second messengers, gene regulators, and mediators for cell activation. But under conditions of excessive production or reduced availability or efficiency of antioxidant mechanisms, ROS may accumulate to unbalanced levels and may then damage almost all cellular components, whether proteins (unfolding, aggregation, desolubilization, and loss of function), lipids (peroxidation of cell and subcellular membranes), DNA, or mitochondria.

Iron, although indispensable to the function of many proteins, including enzymes involved in fundamental processes such as mitochondrial respiration, plays a major role in oxidative stress via Fenton chemistry, where iron(II) is stoichiometrically oxidized by H_2O_2 to iron(III), producing $^{\bullet}OH$ [1]. Thus, among the ROS, the highly reactive and extremely toxic $^{\bullet}OH$ is produced whenever (and wherever) iron is available for the Haber-Weiss reaction ($Fe^{3+} + O_2^{\bullet -} \gg Fe^{2+} + O_2$; $Fe^{2+} + H_2O_2 \gg Fe^{3+} + {}^{\bullet}OH + OH^-$), which is particularly the case in the vicinity of DNA or in the mitochondria; indeed, normal cellular respiration is associated with the production of $O_2^{\bullet -}$ and H_2O_2, thus providing the substrates, in the presence of iron, for $^{\bullet}OH$ generation. Furthermore, iron contributes to redox cycling of cytotoxic compounds such as Adriamycin® or bleomycin, which have a high affinity for DNA. The central role of iron in oxygen toxicity and the ability of iron deprivation to prevent $^{\bullet}OH$ formation by inhibiting the iron-driven Haber-Weiss reaction provides a strong rationale for the use of iron chelators or iron-deficient diets in the prevention of oxidative stress.

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†*Abbreviations*: DMD, Duchenne muscular dystrophy; ROS, reactive oxygen species; IRE, iron-responsive element; IRP, iron regulatory protein; and TNF, tumor necrosis factor.

Endogenously, the availability of iron is tightly regulated within mammalian cells, in particular by the expression of ferritin [2, 3]. Ferritin plays a central role in the cellular protection mechanisms against iron-mediated oxidative injury, and it has been proposed that ferritin should be considered as part of the stress protein families [4]. As other stress proteins, ferritin is ubiquitous and highly conserved. Ferritin specifically binds—"chaperones"—iron, thus protecting cells from the deleterious consequences of iron-catalyzed free radical reactions. Ferritin expression is regulated post-transcriptionally by an iron-dependent mechanism consisting of the *cis*-acting IRE and *trans*-acting IRE binding proteins, IRP1 and IRP2 [2 , 3]. In iron-replete cells, IRP1 fulfills the role of a cytosolic aconitase bound to a 4Fe–4S cluster, while in iron-depleted cells, IRP1 is associated with ferritin mRNA, repressing the translation of ferritin. Transition between the two forms is regulated by the redox status of the cluster. Interestingly, it has been suggested recently that at least part of the beneficial effects of low-dose aspirin in the prevention of atherosclerosis, one of the key manifestations of oxidative stress in humans, could be related to an increase in ferritin synthesis in endothelial cells [5].

IRON AND APOPTOSIS

Apoptosis, from the ancient Greek "falling off" (the tree leaves), is a sophisticated system for programmed cell death and is involved in a number of fundamental biological processes such as metamorphosis, embryonic morphogenesis, development, and removal of superfluous, damaged, or transformed cells. Apoptosis occurs as a counterpart of proliferation in growth regulation and has been associated with pathological processes such as cancer, infections, and chronic inflammation [6–8]. Apoptosis can be induced by a large variety of agents, but, regardless of the inducing agent, the apoptotic process is usually divided into three phases: induction, control, and execution/degradation, all of which have distinct biochemical and morphological features [9–11].

The role of iron in apoptosis remains controversial. In particular, the relationships between ROS, iron, and apoptosis are complex. On the one hand, iron contributes to oxidative stress, a well-characterized inducer of apoptosis [12], and, indeed, iron has, for example, been shown to accelerate endothelial cell apoptosis via a ROS-dependent mechanism [13]. On the other hand, iron is essential for cell growth and viability, and leads to an increased expression of antiapoptotic proteins of the Bcl-2 family, and thus, most likely, in at least partial prevention of apoptosis. It will be of great interest to examine whether iron has the potential to prevent apoptosis in the case of oxidative stress, or whether it potentiates it, via an increased generation of $^{\bullet}OH$. With respect to iron deprivation, most data are in favor of a proapoptotic effect, and it has been established that indeed it leads to the distinctive morphological and biochemical features of apoptosis [9, 10, 14, 15]. The phase(s) of apoptosis in which iron deprivation intervenes is not known, and should also be the subject of further studies.

TAKING AWAY IRON: WHICH DISEASES MIGHT BENEFIT?

Human beings have evolved a critical equilibrium with iron. Iron deficiency anemia affects ≈ 20% of the world's population, and any "therapy by taking away" should be followed up with all necessary care so as not to lead to an aggravation of these numbers. In contrast, the toxic effects of iron in terms of oxidative injury are best exemplified by the pathology of patients with primary or secondary hemochromatosis. Hemochromatosis is the most frequent inherited disease in European populations: homozygosity for the hemochromatosis genes, a recessive trait that leads to iron excess in tissues, occurs in 0.3% of individuals [16], while hemochromatosis secondary to hypertransfusion is an iatrogenic model for iron overload. In both genetically determined and iatrogenic hemochromatosis, the major complication is cardiac failure. The control of iron levels, by exogenous manipulations of iron status, in particular by iron chelators such as desferrioxamine, or more recently IRCO11 [17], or by controlled iron deficiency, can prevent oxidative stress and ROS toxicity.

But the beneficial effects of iron deprivation are not restricted to situations of known iron overload, and iron deprivation also represents an efficient approach for cardioprotection in non-iron-overloaded organisms. A considerable number of studies, whether in rats, rabbits, or dogs, have established the beneficial effects of iron deprivation in ischemia-reperfusion and post-ischemic damage, whether in the brain or the heart [reviewed in Ref. 18], which is in agreement with an ROS-dependent mechanism for reperfusion injury [19].

Furthermore, beneficial effects of iron deprivation have been established or suggested in diseases as diverse as anthracycline cardiotoxicity, organ transplantation, diseases with an inflammatory component such as bleomycin-induced pulmonary fibrosis, bacterial infections and malaria, and, more recently, in DMD [18, 20]. All of these diseases have an oxidative component to their pathogenesis, which suggests that iron deprivation might be beneficial in many other diseases as well. One of the most impressive studies on the beneficial effects of "taking away" reports an 85% prevention of paraplegia secondary to spinal cord injury in swine [21]. Still another example is Alzheimer disease, in which iron also represents an essential source of brain oxidative alterations [22]. Finally, the contribution of iron to the toxic effects of tobacco smoke on atherosclerosis has not been considered before, although iron may mediate a great part of the toxicity of tobacco smoke, whether carcinogenesis or cardiovascular side-effects [23].

IRON IN INFLAMMATION

ROS are among the major mediators of inflammation, and antioxidant therapy has been proposed in many acute or chronic inflammatory diseases, although with limited success. Interestingly, although chronic inflammatory conditions are often associated with iron-deficient anemia, nutritional iron deficiency has been reported to relieve inflammation in a number of these conditions, in particular in adjuvant-induced joint inflammation [24]. Iron-deficient diet has been shown to decrease inflammation significantly in bleomycin-induced pulmonary fibrosis in hamsters [25], a condition mediated by ROS and in particular $^{\bullet}OH$, and by TNFα. Like anthracyclines, bleomycin has high affinity for DNA and enters iron-dependent oxidation-reduction cycles, generating high levels of $^{\bullet}OH$, while at the same time activating NF-κB, as reflected by TNFα release by alveolar macrophages, thus preventing apoptosis of intrapulmonary inflammatory cells.

The outcome of inflammation strongly depends upon apoptosis of the inflammatory cells [8, 11]. Thus, since iron deprivation increases apoptosis, its beneficial effects on inflammation could depend upon dual target mechanisms: prevention of oxidative damage on the one hand, and promotion of apoptosis on the other. Iron deprivation may also exert more specific beneficial effects on the immune response leading to inflammation, such as blocking interleukin-2 receptor expression on T lymphocytes, thus controlling T cell proliferation [26].

IRON AND INFECTIOUS DISEASES

Iron removal may have distinct therapeutic effects in bacterial infections. Indeed, iron is an indispensable nutrient for many bacteria, to the point that bacteria have evolved mechanisms to acquire and secure iron from host cells during host–pathogen interactions [27]. *Mycobacteria*, whether *tuberculosis* or *paratuberculosis*, are paradigmatic for such requirements. The multiplication of the bacteria appears greatly enhanced within iron-replete macrophages, whether murine [28] or human (Boshoff T, Bachelet M, Polla BS and Bornman L, unpublished results). Iron levels of the host thus appear as an essential determinant in the outcome of infectious diseases. In humans, this has been particularly well studied for tuberculosis. Indeed, a relationship between death from tuberculosis and iron overload of the liver and mononuclear-macrophage system (the latter having the strongest association) has been established [29], based on observations from the early twentieth century on iron overload in South African blacks [30]. This condition appears to result from an interaction between the amount of dietary iron and a gene distinct from the HLA-linked iron-loading locus responsible for hereditary hemochromatosis, and has been proposed as an essential component of the increased incidence of tuberculosis in South Africa.

Further supporting a role for iron in bacterial virulence, nutritional iron deficiency attenuates *Salmonella typhimurium* infection in mice as compared with both iron-substituted and normal diet control animals [31]. In malaria, iron deprivation might exert dual beneficial effects, by acting both on the plasmodium itself and on the syndromes caused by the infection, in which ROS-dependent mechanisms again play a key role [32]. In candidiasis, iron deprivation has been shown to restore the antifungal effector functions of phagocytic cells [33].

In the light of these data, although scarce as yet, the possibility that moderate iron deprivation may represent an additional approach for the limitation of pathogenicity and spreading of infectious diseases, including AIDS, should be carefully considered.

IRON DEPRIVATION IN DMD

DMD is a severe genetic disorder due to mutations in the dystrophin gene at Xp21, which prevent the expression of this cytoskeletal, membrane-associated protein in affected individuals, leading to progressive muscle wasting [34]. While the absence of dystrophin is clearly responsible for the DMD phenotype, the exact role of its deficiency in the pathobiochemistry of DMD has remained elusive. One of the approaches taken in several studies to the pathogenesis of DMD has been to consider free radical reactions. Classical antioxidant therapeutic trials, however, have been inconclusive. We thus hypothesized that iron removal may provide a useful starting point for controlling free radical reactions in DMD. This hypothesis was tested in the murine model for DMD, the *mdx* C57BL/10ScSn inbred mice [35], which are genetically homologous to DMD. Although the use of desferrioxamine has been proposed for DMD before [36], we tested dietary iron deprivation as the method of choice for decreasing iron levels, because chelating agents such as desferrioxamine might exert specific effects distinct from iron deprivation. The results were reported by Bornman *et al.* [20]: there is a significant decrease in necrotic fibers in iron-deprived *mdx* mice, which may imply that the iron-driven generation of $^{\bullet}OH$ does indeed play a role in necrosis of dystrophin-deficient muscle fibers and the consequent attraction of inflammatory cells. This reduction in muscle necrosis in the *mdx* mouse following iron deprivation provides a basis for new approaches in the treatment of DMD patients, which is particularly exciting because of the lack of therapy for DMD and the current failure of a timely application of gene therapy. Something as simple as iron deprivation should be examined without delay in other animal models as well as in clinical trials, in all myopathies with an oxidative component including those in which classical antioxidants have not proven efficient [37, 38].

IRON DEPRIVATION AS AN ANTI-AGING FACTOR

Cellular, tissue, and organismal aging has been convincingly associated with a progressive oxidant/antioxidant

imbalance, and decreasing oxidative stress together with increasing antioxidant defenses has provided the source for most *fountains of youth*.

The increased longevity of women as compared with men has been proposed to be related to behavioral components such as a lower exposure to sources of oxidants, including tobacco smoke. I would like to propose here that this evolutionary advantage rather relates to chronic iron deficiency in women during their reproductive life, secondary to menstruation, pregnancies, and deliveries. If indeed oxidative stress and progressive oxidant/antioxidant imbalance is a major component of aging, then iron deprivation might prevent much of the cellular, tissue, and organismal lesions associated with aging and contribute to successful aging. In this perspective, it would be of great interest to examine the iron status of centenarians, along with other indicators of successful aging [39].

Skin aging, a most efficient witness of general premature aging, also presents as an oxidant/antioxidant imbalance, favored by UV radiation exposure [40]. Iron is involved in UV-induced connective tissue degradation and DNA damage [41, 42], and the possibility that moderate iron deprivation might protect skin from UV-induced premature aging and cancer should thus be considered both in experimental animal models and in human cosmetic pharmacotherapy.

If our hypothesis holds true, moderate iron deprivation might shortly add to other anti-aging "miracles" such as fruits and vegetables, supplementation in vitamin E, selenium, and others, and increase individual potential for successful aging, whether skin or organismal aging.

CONCLUSION

In conclusion, therapy by taking away (iron) has a great potential for many very different diseases, all of which share ROS-mediated mechanisms. The development of new, non-toxic, easily administrable iron chelators such as IRCO11 may shortly become the most efficient and fashionable antioxidant, anti-aging, anti-infectious, and anti-inflammatory therapy. In the meantime, although taking away by controlled dietary deprivation is less attractive, it should be considered in all of the above, as well as in the currently incurable, devastating genetic or acquired myopathies such as DMD.

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