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Doxorubicin toxicity in the Iron Age

Clinical Implications

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Anthracyclines, such as doxorubicin, are cardiotoxic. Their administration may result in a refractory form of heart failure; the mechanism is imperfectly defined, although altered intracellular iron metabolism appears to play a role. In this issue, Corna et al. investigated the notion that iron regulatory protein 1 (IRP1) might mediate doxorubicin toxicity [1]. They gave doxorubicin to wild-type and IRP1 gene-deficient mice. The mice down-regulated iron regulatory protein 2 (IRP2) but got sick anyway. The authors conclude that doxorubicin toxicity occurs independently of IRB1. Their precise electrophoretic mobility shift assays, Western blots, Northern blot analyses, oxidative stress estimates, and brain natriuretic peptide read-outs convince the hardened basic scientist, not to mention the naive clinician. In terms of doxorubicin toxicity, we remain the oxen standing in front of the mountain; we have not gotten very much smarter. However, perhaps we clinicians, who after all must prescribe the anthracyclines, should have another look at doxorubicin toxicity, the IRP molecules, and their function.

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All mammalian cells require iron for oxygen delivery or as an enzymatic transfer element [2, 3]. However, excess iron promotes the formation of oxygen radicals that attack cellular lipids, proteins, and nucleic acids. Iron homeostasis is a closed box; in contrast to sodium, calcium, and magnesium, we do not pee or poop it out. Thus, intestinal iron uptake is carefully regulated and only a minor fraction of the supply is translated into demand. Iron cannot pass lipid bilayers without carrier proteins. Once absorbed, iron circulates bound to transferrin, an abundant plasma protein that binds two iron atoms with high affinity. Iron is delivered to transferrin receptors. The homodimer transferrin receptor binds two molecules of transferrin and its iron load and by clathrin-mediated invagination moves the complex to an endosome. Inside the endosome, proton pumps, resulting in conformational changes of both the transferrin and transferrin receptor proteins, lower pH. Iron is released and reduced to the Fe^{2+} form. The divalent metal Transporter I, a proton-dependent iron carrier with 12 membrane-spanning segments, then moves Fe^{2+} across the endosomal membrane into the cytoplasm. In the cytoplasm, iron is either incorporated into protoporphyrin to produce heme or retained in storage forms. Meanwhile, the transferrin and the transferrin receptor are returned to the cell surface for use elsewhere. This, inexcusably oversimplified, probably inaccurate, description was the easy part.

The expression of transferrin receptor (s) and ferritin, the shell-like iron storage protein, is coordinated and reciprocally controlled in response to iron supply at the posttranscriptional level [4]. The mRNAs encoding transferrin receptor and ferritin contain structural motifs known as iron responsive elements (IREs) in their untranslated regions (UTRs). These are hairpin structures that consist of about 30 nucleotides and are highly conserved. The IREs become targets of two cytoplasmic iron regulatory proteins named (you guessed it) IRP1 and IRP2. The IRPs bind to their targets with high affinities and thereby stabilize an otherwise unstable mRNA. As a result, iron-starved cells increase their capacity to take up transferrin-bound iron by the transferrin receptor and minimize iron sequestration into ferritin stores. Conversely, in iron-replete cells, IRP1 and IRP2 fail to bind to cognate IREs, thereby permitting transferrin receptor mRNA degradation and ferritin mRNA translation. This response inhibits further iron uptake and promotes the storage and detoxification of excess iron.

IRP1 and IRP2 are homologous cytoplasmic polypeptides of 889 and 964 aminoacids, respectively, and belong to the iron–sulfur cluster isomerases [4]. Both proteins have been deleted in the mouse. IRP1-deleted mice had no obvious phenotype, while IRP2-deleted mice have aberrant iron homeostasis and accumulate the metal in the gastrointestinal tract and the brain. Thus, IRP2 seems to be the important regulator of systemic iron metabolism, which is in accord with reports on IRP2 polymorphisms and human disease. IRP1 is

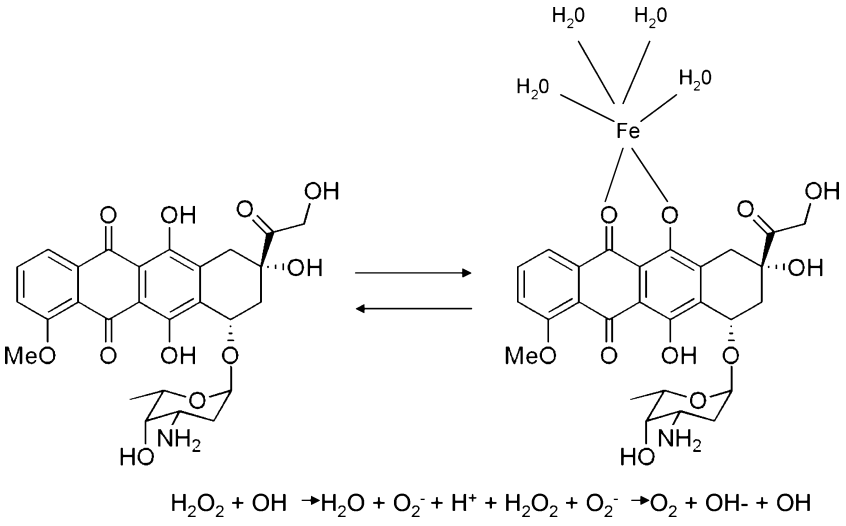

Fig. 1 One-electron reduction of doxorubicin generates the doxorubicin-semiquinone that induces DNA damage and lipid peroxidation by reactive oxygen species generation. In the *lower portion*, the famous Haber–Weiss reaction is shown. Fritz Haber would have been fascinated as to the clinical implications of his work (see 5 and Fritz Haber's papers)

regulated by means of an unusual iron–sulfur cluster switch, while IRP2 is regulated in response to iron and oxygen supply. If you guessed that HIF-1alpha could play a role here, you are correct. Even nitric oxide seems to get into the act. However, now what about the anthracyclines?

The main anticancer anthracycline antibiotic is doxorubicin. Doxorubicin has several toxic actions. It binds to DNA and inhibits both DNA and RNA synthesis; however, doxorubicin's main cytotoxic action appears to be mediated through an effect on topoisomerase II, a DNA gyrase that is markedly increased in proliferating cells. During replication of the DNA helix, reversible swiveling must take place around the replication fork to prevent the daughter DNA molecule from becoming inextricably entangled during mitotic segregation. Topoisomerase II provides the swivel. Doxorubicin intercalates in the DNA and causes the process to seize up. Aside from all that, anthracyclines bind avidly to iron, forming a 1:1, 2:1, or 3:1 drug-to-metal complex [5]. Doxorubicin can bind directly to iron and in the presence of oxygen, the drug can cycle between the Fe^{2+} and Fe^{3+} states (Fig.1). The doxorubicin-Fe^{3+} can be reduced to doxorubicin-Fe^{2+} by NADPH cytochrome P450 reductase, glutathione, and cysteine. These reactions are accompanied by the formation of superoxide and the conversion of anthracycline quinone moieties to semiquinone free radicals. The quinone structure of anthracyclines can act as an electron acceptor from flavin reductases, NADH dehydrogenase, and cytochrome P450 reductase. The iron-catalyzed Haber–Weiss reaction, H_2O_2, and extremely reactive hydroxyl radicals are generated (Fig.1). The semiquinone radical may form an aglycone radical, which is a potent alkylating agent. Reactive oxygen species generation by anthracyclines leads to DNA damage and apoptosis. Cardiac tissue is particularly vulnerable to free radical damage because of a low antioxidant enzyme system activity.

Doxorubicin has been implicated in both IRP1 and IRP2 homeostasis [5]. Doxorubicinol is a secondary alcohol metabolite of doxorubicin. Doxorubinicol interacts with the 4Fe–4S cluster of IRP1, resulting in the release of Fe^{2+} and a decrease in cytoplasmic aconitase activity. Decreased cytoplasmic aconitase levels are said to increase IRP–RNA binding. However, doxorubicin inactivates both IRPs in cardiomyocytes exposed to the drug by distinctly different mechanisms [6]. The data presented by Corna et al. [1] show that doxorubicin caused IRP2 down-regulation, increased ferritin expression, and decreased transferrin receptor expression. When IRP1 was absent, the same responses were observed. The authors suggest that the reduction of cardiac IRP2 activity might represent a protective mechanism to limit doxorubicin toxicity. However, IRP2 down-regulation was not sufficient to protect either wild-type or IRP1 gene-deficient mice. The authors argue that studying IRP2 gene-deficient mice would not necessarily answer the question of whether or not IRP2 down-regulation provides protection. I would, nevertheless, have been curious if IRP2 gene-deleted mice are less susceptible to doxorubicin-induced cardiac damage.

The phenotypes of these deleted mouse strains are by no means similar [7, 8]. IRP1 gene-deleted mice misregulate iron metabolism only in the kidney and brown fat, two tissues in which the endogenous IRP1 expression greatly exceeds that of IRP2. IRP2 gene-deleted mice in contrast have misregulated target protein expression throughout the body. IRP2 gene-deleted mice develop a movement disorder at 6 months into adulthood, related to ferric iron accumulation in the cytosol of neurons and oligodendrocytes. The RNA-binding activity of IRP1 does not increase in animals given a low-iron diet that is sufficient to activate IRP2. IRP1 appears to be bifunctional and exists commonly in the form of cytosolic aconitase, rather than as an RNA-binding protein. The IRP knockout experiments indicate that the small RNA-binding fraction of IRP1 that is insensitive to cellular iron status, contributes to basal mammalian iron homeostasis, whereas IRP2 is sensitive to iron status and can compensate for the loss of IRP1 by increasing its binding activity. Thus, IRP2 appears to dominate post-transcriptional regulation of iron metabolism in mammals. Corna et al. suggest that expressing a doxorubicin insensitive form of IRP2 in the heart would elucidate the role of IRP2 downregulation, as would no longer modulate ferritin or transferrin receptor expression and should have increased cardiac damage [1].

In addition to the above, doxorubicin influences iron trafficking pathways. Early studies suggested that doxorubicin released iron from ferritin. More recent work suggests that doxorubicin increases intracellular ferritin-iron levels three- to eightfold, compared to control cells [5]. The

anthracycline apparently interfered with iron release to form the protein. Catabolism of ferritin by lysosomes presumably subsequently occurs. Anthracyclines may accumulate in lysosomes, thereby interfering with their function. In any event, doxorubicin cardiotoxicity remains an enigma.

An important clinical question is whether or not doxorubicin cardiotoxicity can be prevented. Dexrazoxane is the current front-runner. Dexrazoxane permeates cell membranes and serves as a metal ion-binding metabolite, thereby decreasing doxorubicin iron-binding and reactive oxygen species production. Desferrioxamine is a hexadentate iron chelator used for iron-overload conditions. The material is poorly tolerable and the difficulties appear to exceed the putative advantages. Other iron chelators have been tried. Probucol, amlodipine, melatonin, and even garlic are in the running. In any event, there is much to do [9].

With this editorial, I complete 10 years as pundit for J Mol Med. I am not certain that I have provided the readership with any additional insights. However, I am thoroughly convinced that I have learned an incredible amount by attempting to garner some expertise on a heterogeneous subject material. I thank you for bearing with me.

Respectfully,
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