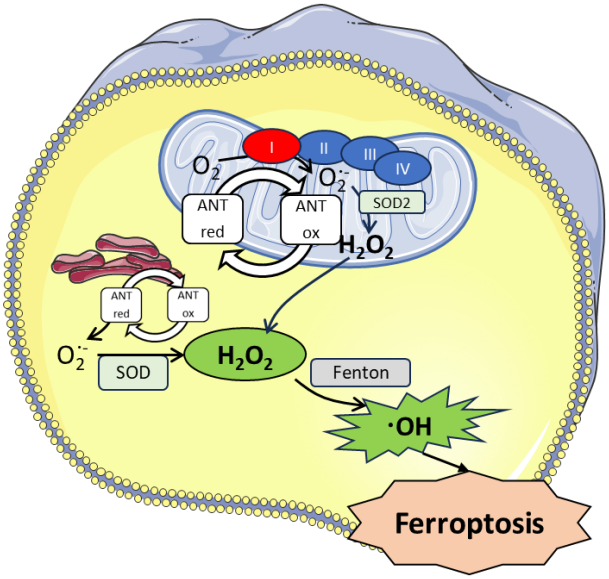
Hydroxyl Radical, the dark messenger connecting Ferroptosis and Doxorubicin-Induced Cardiotoxicity (DIC)

Unveiling hydroxyl radical as a missing link hidden in H_2O_2, its Trojan horse.

The interplay between ferroptosis and doxorubicin (DOX) has recently emerged as a relevant area of research, as it helps to explain the mechanisms behind doxorubicin-induced cardiotoxicity (DIC), cellular oxidative stress and programmed cell death. Unveiling the role of ROS species in these biological mechanisms, with a specific focus on the hydroxyl radical (•OH), potentially opens the door to novel therapeutic targets and treatments.



1. Overview of Doxorubicin and Cardiotoxicity

Drugs that were initially approved for medical use have been recalled from clinical use due to long-term toxicity discovered after they were widely prescribed. Cardiotoxicity and cardiovascular safety (aka drug-induced cardiotoxicity) are the third most common reason for adverse drug reactions and accounted for 10-14% of withdrawals in the last four decades, including previously successful therapeutics such as Rofecoxib (Vioxx), Tegaserod (Zelnorm/Zelmac) and Sibutramine (Meridia).

Doxorubicin (DOX) is a powerful chemotherapeutic agent used to treat various cancers. However, its clinical use is limited by its severe side effects, particularly cardiotoxicity, which can lead to irreversible heart damage and heart failure.

The proposed main mechanism of **doxorubicin-induced cardiotoxicity (DIC)** is increased oxidative stress, as evident from increased levels of reactive oxygen species, mitochondrial dysfunction and lipid peroxidation, which ultimately results in cell death of cardiomyocytes (heart muscle cells). Nevertheless, several novel molecular targets and signalling pathways underlying DIC have emerged over the past few years, such as impairment of calcium homeostasis, autophagy and epigenetic alterations at DNA and histone levels. The most notable advances include discovery of ferroptosis as a major form of cell death by DOX.

2. Mechanism of Ferroptosis

Ferroptosis is a form of regulated cell death distinct from apoptosis, necrosis, and autophagy. It is characterized by iron dependence (ferroptosis is driven by iron accumulation in cells), lipid peroxidation (phospholipid peroxides leads to membrane damage and cell death), glutathione depletion (a major cellular antioxidant), and the inactivation of glutathione peroxidase 4 (GPX4, an enzyme that prevents lipid peroxidation).

Although it is still intriguing whether ferroptosis plays a physiological role in healthy cells (e.g. tumour suppression and immune surveillance), it is accepted that it stems from an imbalance in the exquisite cellular mechanisms of oxidative defence, thus its role in disease and therapy.

3. How Doxorubicin Induces Ferroptosis: the missing links

ROS Generation:

DOX generates reactive oxygen species (ROS) which increase oxidative stress in cardiomyocytes. Anthracyclines, e.g. DOX, are prone to redox cycling at endoplasmic reticulum and mitochondria, generating superoxide anion radicals (O_2·-) from the reduction of molecular oxygen through either enzymatic catalysis by NADPH-P450 reductase, xanthine oxidase (XO) or NOS , or direct reaction with oxygen or oxidized glutathione. Moreover, DOX increases the activity of NADPH oxidase (Nox), which also generates O_2·-. Superoxide dismutase (SOD) converts O_2·- into hydrogen peroxide (H_2O_2), which eventually generates hydroxyl radical (·OH) by

Fenton reaction with Fe2+ iron. Hydroxyl radical is the most reactive ROS species and rapidly reacts with molecules around in close proximity, such as DNA, proteins or phospholipids. Remarkably, phospholipid peroxidation leading to membrane damage is the hallmark of ferroptosis, a programmed cell death pathway different to apoptosis. The lifetime of hydroxyl radicals is in the nanosecond range, so this species of ROS cannot travel very far inside the cell. In contrast, H_2O_2 is hardly reactive and remains stable in cells unless degraded by enzymatic systems, such as catalase, MPO, Gpx and Trx. Furthermore, H_2O_2 can traverse lipid membranes and reach virtually any site to distribute its toxic effect. **Hydroxyl radical plays the role of dark messenger hidden in H_2O2, its Trojan horse.**

Iron Metabolism Disruption:

- DOX disrupts iron homeostasis by increasing the intracellular labile iron pool (LIP). This is achieved by enhancing iron import (via transferrin receptor) and reducing iron storage and export (e.g., downregulating ferritin and ferroportin).
- The elevated iron levels in the presence of ROS lead to the Fenton reaction, producing highly reactive hydroxyl radicals, which exacerbate lipid peroxidation.

Lipid Peroxidation:

- The oxidative environment created by DOX, combined with excess iron, promotes the peroxidation of polyunsaturated fatty acids (PUFAs) in cellular membranes. Lipid peroxides are the key mediators of ferroptosis, causing membrane damage and cell death.

GPX4 Inactivation and Glutathione Depletion:

- DOX-induced oxidative stress depletes glutathione levels, weakening the cell's ability to combat lipid peroxidation. This results in the inactivation of GPX4, a crucial enzyme that prevents ferroptosis by reducing lipid peroxides to non-toxic alcohols.
- The loss of GPX4 activity is a tipping point that leads to the accumulation of lethal lipid peroxides, culminating in ferroptosis.

Nrf2 inhibition:

- Several studies have demonstrated that doxorubicin has a heightened sensitivity to oxidative stress indicators, specifically the inhibition of antioxidant signalling pathways such as Nrf2 and its downstream antioxidant factors HO-1 and NQO1. This inhibition leads to increasing levels of ROS in the cell, which stimulates mitochondrial membrane damage in cardiomyocytes and production of apoptotic bodies, resulting in DNA fragmentation and eventually apoptosis in cardiomyocytes.

4. Evidence of Ferroptosis in DIC: Therapeutic Implications

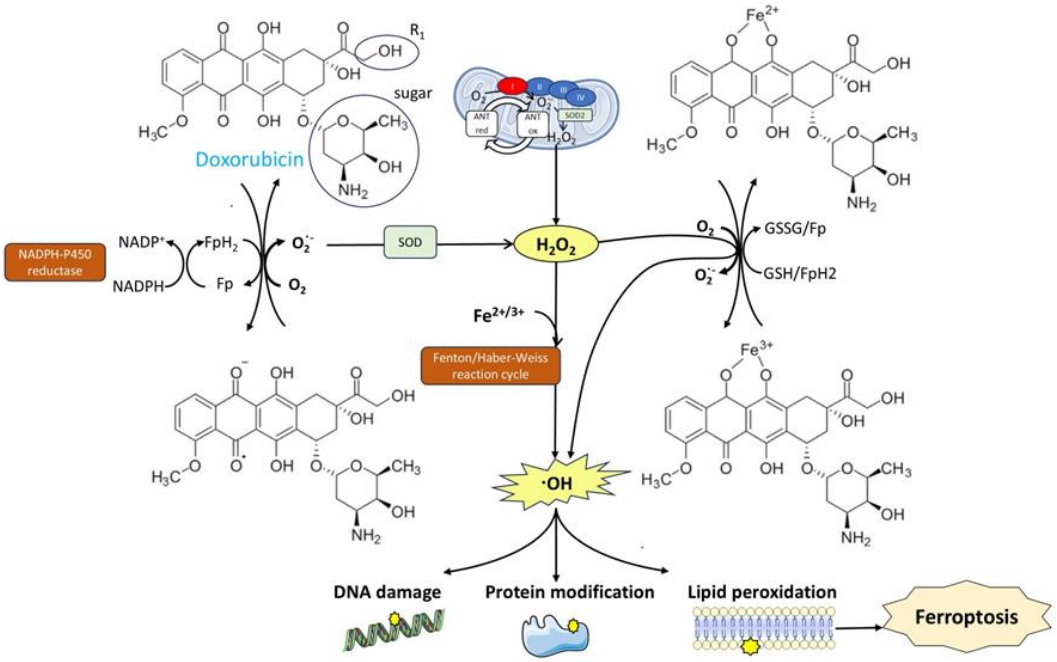
- Recent studies have demonstrated that markers of ferroptosis, such as lipid peroxidation products and iron accumulation, are elevated in cardiomyocytes treated with DOX.
- Genetic or pharmacological inhibition of ferroptosis (e.g., using ferrostatins or liproxstatins) has been shown to protect against DOX-induced cardiotoxicity, highlighting the role of ferroptosis in this process.
- The use of iron chelators like dexrazoxane, which sequesters free iron and prevents the Fenton reaction, has been shown to reduce DOX-induced cardiotoxicity. This supports the idea that iron-mediated ROS production and ferroptosis are central to the damage caused by DOX.

5. Therapeutic Implications

Doxorubicin (DOX) causes a dose-dependent cardiotoxicity manifested by cardiac enlargement, ventricular dilation, interstitial fibrosis, and decline of left ventricular ejection fraction (LVEF). Ferroptosis serves as a core mechanism and participates in the progression of DOX-induced cardiotoxicity. Combining DOX with ferroptosis inhibitors or antioxidants may allow for more effective cancer treatment with reduced cardiac side effects, improving the overall safety and efficacy of DOX-based chemotherapy. In this context, multiple natural and pharmaceutical compounds have been shown to inhibit DOX-induced ferroptosis and cardiomyopathy. Potential therapeutic strategies include iron regulators reducing iron availability, inhibitors of phospholipid peroxidation, radical-trapping antioxidants (RTAs), nuclear factor-erythroid 2-related factor 2 (Nrf2) activators, agents which preserve GPX4 activity and adenosine monophosphate-activated protein kinase (AMPK) activators. On the other hand, ferroptosis inducers exhibit antitumoral potential as sensitizers of cancer cells.

6. The relevance of detecting hydroxyl radicals in cells

As aforementioned, hydroxyl radical (¤OH) plays the pivotal role of dark messenger hidden in H2O2, its Trojan horse, translating the action of oxidative stressors (e.g. DOX) into programmed cell death (e.g. ferroptosis). Since its extremely low lifetime and high reactivity, selective and primary detection of hydroxyl radical turns to be challenging, thus neglected in



many cell biology studies. Monitoring of hydroxyl radicals allows establishing a reliable link between primary signals and downstream phenotypes. A4cell has developed **CytoCHECK SPAchip® OHrad ROS Single-Detection Kit**, a groundbreaking sensor for the selective detection of hydroxyl radicals (•OH) using silicon chips and fluorescence microscopy in live cells. The kit is insensitive to pH and only active when processed by intracellular esterases (Read more).

radicals in particular, and ferroptosis in this process, new therapeutic strategies can be developed to protect the heart without compromising the anti-cancer efficacy of doxorubicin. This understanding not only deepens our knowledge of doxorubicin's side effects but also opens new avenues for targeted therapies that could greatly enhance the quality of life for cancer patients undergoing treatment.

7. Conclusions

The link between doxorubicin and ferroptosis is an important area of research, with significant implications for understanding and mitigating doxorubicin-induced cardiotoxicity. By elucidating the role of ROS, and hydroxyl

CytoCHECK SPAchip® OHrad ROS Single-Detection Kit enables the effective and selective monitoring of hydroxyl radical (·OH) in live cells

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