



Review

Where ferroptosis inhibitors and paraquat detoxification mechanisms intersect, exploring possible treatment strategies



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ABSTRACT

Paraquat (PQ) is a fast-acting and effective herbicide that is used throughout the world to eliminate weeds. Over the past years, PQ was considered one of the most popular poisoning substances for suicide, and PQ poisoning accounts for about one-third of suicides around the world. Poisoning with PQ may cause multiorgan failure, pulmonary fibrosis, and ultimately death. Exposure to PQ results in the accumulation of PQ in the lungs, causing severe damage and, eventually, fibrosis. Until now, no effective antidote has been found to treat poisoning with PQ. In general, the toxicity of PQ is due to the formation of high energy oxygen free radicals and the peroxidation of unsaturated lipids in the cell. Ferroptosis is the result of the loss of glutathione peroxidase 4 (GPX4) activity that transforms iron-dependent lipid hydroperoxides to lipid alcohols, which are inert in the biological environment. Impaired iron metabolism and lipid peroxidation are increasingly known as the driving agents of ferroptosis. The contribution of ferroptosis to the development of cell death during poisoning with PQ has not yet been addressed. There is growing evidence about the relationship between PQ poisoning and ferroptosis. This raises the possibility of using ferroptosis inhibitors for the treatment of PQ poisoning. In this hypothesis-driven review article, we elaborated how ferroptosis inhibitors might circumvent the toxicity induced by PQ and may be potentially useful for the treatment of PQ toxicity.

1. Introduction

Paraquat (PQ) is a non-selective, inexpensive, efficacious, and environmentally benign herbicide (Cui et al., 2018). These properties lead to its extensive application in many countries around the world. The first poisoning from this agent in humans was reported in 1966 (Bullivant, 1966), and also annually, it causes accidental and deliberate

poisoning worldwide (Qing-Feng et al., 2012). It has been shown that PQ poisoning results in severe damage to kidneys, lungs, brain, and liver as well as other organs (Shadnia et al., 2018). Studies demonstrated that PQ poisoning is associated with complications such as acute lung failure, pulmonary hypertension, leukocytosis, metabolic acidosis, enlargement of heart, acute damage to the kidney, diffusion edema, and increased level of amylase, blood sugar, and creatinine (Delirrad et al.,

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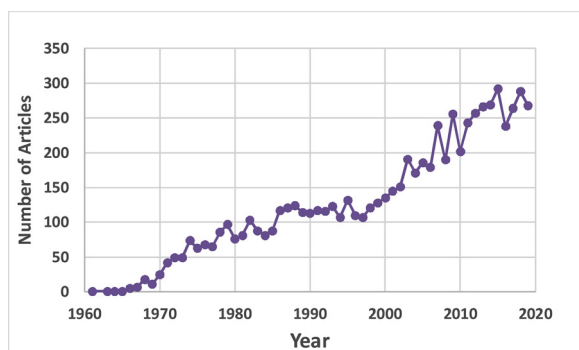


Fig. 1. The number of articles in the PubMed database of NCBI with the keyword “paraquat”.

2015). The following chart describes how medical attention toward paraquat is raised based on the number of PubMed articles each year (Fig. 1).

D'Souza et al. suggested that PQ is genotoxic and cytotoxic for male germ cells (D'Souza et al., 2006). Because of severe lung damage in acute exposure to the high dose of PQ and according to the recent reports which revealed its genotoxic nature and involvement in skin cancer and Parkinson's disease (in long-term exposure to low dose of PQ), this herbicide received critical considerations in medicine and toxicology (Wesseling et al., 2001).

So far, no effective and safe antidote has been found for the treatment of PQ poisoning (Dinis-Oliveira et al., 2008). Regardless of the treatment, intoxication with PQ leads to death following several days in most patients due to multiorgan failure resulting from a decreased number of cells due to cell death. In various experimental models, cell death due to exposure to high doses of PQ by means of apoptosis has been suggested (Yamada et al., 2015). Surprisingly, in a study by Hirayama et al., it was shown that cell death in SH-SY5Y cell line caused by PQ should be complex and cannot be fully explained by known mechanisms (Hirayama et al., 2018).

2. Pathophysiology of paraquat toxicity

The distribution volume of PQ is high (1.2–1.6 L/kg) and has the ability to accumulate in organs and tissues such as kidney and lung (Gawarammana and Buckley, 2011). PQ accumulates in the lungs principally by active transfer and polyamine transporters. These polyamine transporters are mainly involved in the transportation of polyamines like spermine, spermidine, putrescine (Pegg, 2009). PQ concentration in the lungs is ten times higher compared to the plasma. Cellular uptake in the lung is performed by type I and II alveolar epithelial cells through the uptake route of polyamines. Cellular uptake of PQ occurs due to structural similarity between PQ and androgen diamines and polyamines, including putrescine and spermidine. Studies have demonstrated that PQ is accumulated selectively in lung tissue and results in remarkable tissue destruction and fibrosis in the lung (Chen, 2015; Liu et al., 2018; Smith, 1987).

The exact mechanisms involved in the uptake of paraquat is not fully elucidated. It is thought that there are some active transport mechanisms because the accumulation observed was against the PQ concentration with saturation kinetics. It was not explained which type of receptor this is and no recent study is accomplished to reveal the exact mechanism behind this. Therefore, it is not clear whether the polyamine transporters are involved in the active transport or other carriers are engaged in this accumulation, and even how much this active transport is actually involved in PQ entrance into the lung cells. ABC transporters are known to excrete paraquat actively from plant cells and cause paraquat resistance, but the impact of these transporters on human toxicity is not explained (Moretti et al., 2017; Xi et al., 2012).

ABC transporters typically expel the substrates out of the cell and are involved in drug resistance (Jabbarzadeh Kaboli et al., 2019); therefore it is unlikely that they are responsible for the active accumulation observed for PQ. There are some cases indeed, in which ABC transporters contributed to the accumulation of xenobiotics (Declèves et al., 2008), but the significance of these proteins for PQ distribution in human (lung) cells requires further exploration. On the contrary, high levels of p-glycoprotein (P-gp) were related to reduced lung concentrations of PQ in rats and lower toxicity toward human epithelial colon cancer cells (Wu et al., 2018).

Also, an active tubular secretion is identified for PQ, because the total amount of excreted PQ cannot be merely described by glomerular filtration rate (GFR). Indeed, “quinine and N-methylnicotinamide (NMN) reduced the fractional excretion of PQ, suggesting that they share the same cation transport system with PQ” (Dinis-Oliveira et al., 2008).

Previous studies advocate the idea that ferroptosis plays a decisive role in pulmonary fibrosis, and ferroptosis inhibitors prevent fibrosis (X. Li et al., 2019a). All the phenotypical characteristics of pulmonary fibrosis induced by TGF-β1 and erastin (a ferroptosis inducer) were recovered using ferrostatin-1 (a ferroptosis inhibitor) (Gong et al., 2019). Induction of fibrosis can also be accompanied by ferroptosis in other tissues like the liver (Wang et al., 2019) and heart (Fang et al., 2019).

PQ stimulates the synthesis of Reactive Oxygen Species (ROS) and subsequently culminates in cellular damage through lipid peroxidation, NF-κB activation, mitochondrial damage, and apoptosis in many organs. The tissue damage provoked by PQ is associated with cellular oxidant/antioxidant imbalance. PQ is metabolized by several enzymatic systems, including NADPH-cytochrome P450 reductase, xanthine oxidase, NADH-ubiquinone oxidoreductase and nitric oxide synthase (Gawarammana and Buckley, 2011). After the entrance of PQ to the cells, it is reduced to PQ²⁺ via the electron donor NADPH to yield PQ⁺, which is also called paraquat free radical (Kim et al., 2011). PQ⁺ is oxidized by oxygen and then returns to its primary form (PQ²⁺), and during this process, superoxide radical is produced (Dinis-Oliveira et al., 2008; Gawarammana and Buckley, 2011). Other ROS such as hydroxyl radicals may be produced via Haber-Weiss reaction (Fig. 2). This reaction is very slow, but iron and other metal-chelating ions can catalyze this reaction, which is called “Fenton reaction” (Lipinski, 2011; Kehrer, 2000; Winterbourn, 1995).

When the production of free radicals enhances, lipid peroxidation occurs, and finally, the structure of cell membrane alters (Ayala et al., 2014). Generally, PQ toxicity is linked to the overproduction of ROS and the induction of cellular oxidative damages through lipid peroxidation. Lipid peroxidation leads to deleterious effects such as osmotic fragility, decreased mitochondrial survival and reduced membrane fluidity (Cazzola et al., 2004). In fact, hydroxyl radical is one of the most common types of ROS, which primarily affects unsaturated lipids

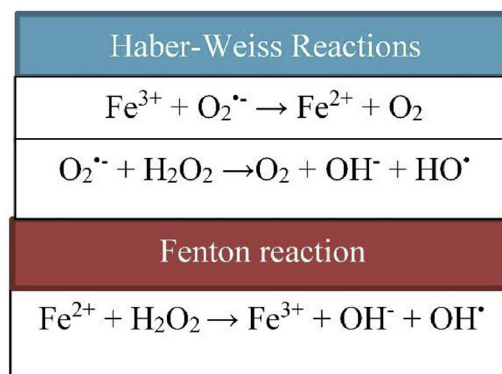


Fig. 2. Haber-Weiss Reactions and Fenton reaction, a major route for induction of ROS and ferroptosis.

and is also involved in membrane damage through lipid peroxidation during exposure to PQ (Ayala et al., 2014). Recently, Hou et al. demonstrated that PQ, by means of NADPH oxidase, results in neurodegeneration of dopaminergic neurons through ferroptosis (Hou et al., 2019). The authors of the study suggested anti-ferroptotic therapy for the treatment of pesticide-induced neurotoxicity, particularly for PQ and maneb.

3. Ferroptosis and lipid peroxidation

Lipid peroxidation process can be triggered by some oxidants such as H_2O_2 , superoxide, reactive hydroxyl radicals during pathological conditions, and by exposure to xenobiotics and environmental contamination. Lipid peroxidation can impair the structure and function of the cell membrane, and if this process is not controlled, it could lead to the impairment in cellular function and tissue damage (Ramana et al., 2017).

The current definition of ferroptosis is the cell death process promoted by lipid peroxidation, which can be inhibited either by iron chelators or lipophilic antioxidants (Feng and Stockwell, 2018). Ferroptosis is involved in many human pathologies and treatment strategies. Recent evidence has shown the role of ferroptosis in a variety of degenerative kidney, liver, and brain diseases such as Parkinson's, Alzheimer's, and Huntington's diseases as well as traumatic and hemorrhagic injuries (Stockwell et al., 2017). There are several distinct proteins involved in the regulation of ferroptosis. Glutathione peroxidase 4 (GPX4), Nuclear factor erythroid 2-related factor 2 (Nrf2), Metallothionein-1 G (MT1G), Heat shock protein β -1 (HspB1) are some of the negative regulators of ferroptosis. By contrast, NADPH oxidase, MAPK, PKC α , and p53 serve as positive regulators of ferroptosis by enhancing ROS production, repressing the expression of SLC7A11, or even through some complex signaling cascades (Ding and Yin, 2017).

Ferroptosis is distinct from other cell death types based on morphological and biochemical properties and is distinguished by the aggregation of lipid peroxides (Gaschler and Stockwell, 2017). GPX4 repairs the oxidative damage of the cell membrane and eliminates the dangerous oxidized products emanated from lipid peroxidation by iron. The precise mechanism underlying the initiation of ferroptosis upon the accumulation of lipid peroxides is an active area of investigation (Forcina and Dixon, 2019). Ferroptosis is promoted by inhibition of cysteine uptake or inactivation of GPX4 for fat repair. Additionally, this process can be instigated through chemical or mutational inhibition of cysteine/glutamate antiporter (SLC7A11), and also increased accumulation of ROS accumulate in the form of lipid hydroperoxides (Latunde-Dada, 2017). For instance, a high concentration of extracellular glutamate results in the inactivation of SLC7A11 and the reduction of cysteine uptake. This would finally deplete the glutathione (GSH) reservoir of the cells and leads to ferroptosis (Nagata and Nakano, 2017). Thus, there are four major pathways for initiation of ferroptosis:

- 1) Inhibition of SLC7A11 (also known as system Xc⁻ or cystine/glutamate transporter);
- 2) Direct inhibition of GPX4;
- 3) Depletion of GPX4 protein and CoQ10;
- 4) Induction of lipid peroxidation (Feng and Stockwell, 2018).

Ferroptosis is manifested by a) overproduction of ROS; b) Depletion of GPX4 in cells; c) accumulation of lipid hydroperoxides; and d) iron overloading (Bertrand, 2017). Iron metabolism and lipid peroxidation are known as major mediators of ferroptosis. Besides, activation of MAPK signaling may prompt ferroptotic cell death of cancer cells (Y. Xie et al., 2016a). Overproduction of ROS also leads to the sensitization of cells to ferroptosis via the repression of SLC7A11 transcription, which is mediated by p53 (Ou et al., 2016).

Erastin and RSL3 are two small molecules of ferroptosis inducers as an initial discovery, which both exert their deadly effects by evacuating the antioxidant capacity of the cells. Erastin inhibits SLC7A11, a transmembrane cystine/glutamate antiporter that provides cysteine for

the synthesis of the antioxidant tripeptide GSH. RSL3 inactivates GPX4 to induce ROS production from lipid peroxidation (Gaschler and Stockwell, 2017).

The current data suggests a model that lipoxygenases (LOXs) and non-enzymatic Fenton reaction contribute to the lipid peroxidation during ferroptosis (Feng and Stockwell, 2018). Apparently, ferroptosis is mainly induced by reducing the detoxification of lipid peroxides by GPX4 or by draining GPX4's capacity. Furthermore, the production of polyunsaturated fatty acids (PUFAs) is a primary marker of ferroptosis. Oxidation of PUFAs by LOXs results in the accumulation of lipid peroxidation products (Latunde-Dada, 2017). Indeed, GPX4 inhibition, GSH diminution, and increased level of LOXs' activity cause the augmented accumulation of PUFA hydroperoxides and production of fatty acids such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) and finally trigger ferroptosis in pathological conditions (Ayala et al., 2014; Feng and Stockwell, 2018).

It has been suggested that PQ induces the activation of the inflammasome and subsequent inflammatory pathways related to inflammation via NLRP3, which provokes lung injury (Liu et al., 2014). Free heme and labile iron in the cellular environment have been shown to produce inflammation by activation of NLRP3 (Erdei et al., 2018). Activation of the inflammasome is linked with many pathological conditions such as cancer, cardiovascular diseases, autoimmune disorders, and neurodegenerative impairments (Moossavi et al., 2018; Zhang et al., 2017).

In recent years, different studies have regarded ferroptosis as a defense mechanism against cancer, neurotoxicity, and injury due to ischemia or reperfusion. In the study by Alvarez et al., inhibition of NFS1 expression could induce ferroptosis and suppress lung tumor growth (Alvarez et al., 2017). In addition, Dixon et al. found that ferrostatin-1 (as a small molecule inhibitor of ferroptosis) specifically inhibited ferroptosis in cancer cells and prevented neuronal toxicity from post-birth glutamate in rat brain (Dixon et al., 2012). Despite the aforementioned documentations, there are no studies concerning the role of ferroptosis in cell death during PQ toxicity. Therefore, unraveling the role of ferroptosis in cell death caused by PQ toxicity provides a new perspective for PQ toxicity and its treatment strategies.

4. Potential novel treatment strategies by ferroptosis inhibitors

Ferroptosis inhibitors with antioxidant properties are one of the potential compounds for overwhelming the toxicity induced by PQ. They restore the reduction-oxidation balance of the cells and inhibit the cell death caused by ferroptosis. Some of them might have low or no antioxidant activity, but inhibition of ferroptosis seems to be the main or at least one of the main mechanisms involved in their PQ detoxification. Recent findings suggest that some other proteins (like N-methyl-D-aspartate receptor, NMDAR), which are indirectly responsible for maintenance of the oxidation-reduction cycle, might be potential therapeutics for the treatment of PQ poisoning, which are discussed in the next section (Fig. 3).

4.1. Deferoxamine

Iron chelators are one of the ferroptosis inhibitors which prevent lipid peroxidation via the inhibition of LOXs and diffusion of lipid peroxidation products by Fenton reaction suppression. Deferoxamine is a drug that is used for acute iron toxicity and hemosiderosis and is also suitable for *in vivo* studies (Di Nicola et al., 2015; Feng and Stockwell, 2018). It seems that iron and PQ have synergistic effects in the production of hydroxyl radicals in Fenton reaction associated with iron. However, according to the *in vitro* studies, it has been suggested that the presence of PQ results in the acceleration of Fe³⁺ reduction to the Fe²⁺ ion. So, there is advancement in the production of hydroxyl radical until a sufficient amount of H₂O₂ is available. Fe³⁺ reduction might occur either directly via PQ²⁺ or indirectly from radical superoxide

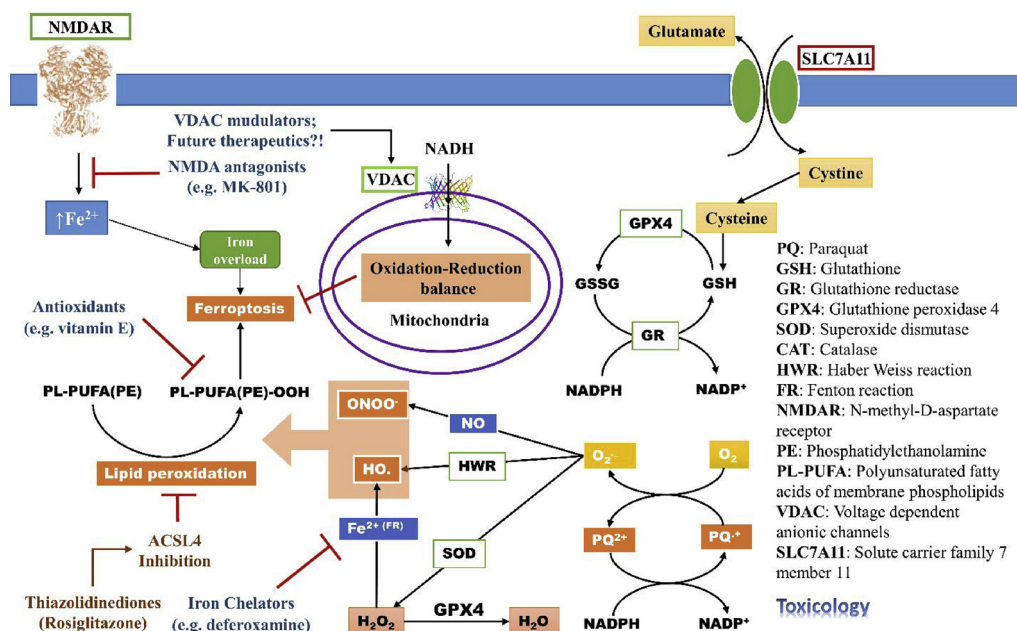


Fig. 3. Possible mechanisms that depict how ferroptosis inhibitors could be used for the treatment of PQ toxicity (some pathways are not depicted due to their complexity).

production through oxygen reduction by PQ^{2+} . The importance of iron and other transition metals in PQ damages has been investigated by *in vitro* and *in vivo* studies. It is well documented that iron chelators prevent PQ toxicity (Kohen and Chevion, 1985; Korbashi et al., 1986; Van Asbeck et al., 1989; van der Wal et al., 2011). It has been reported that deferoxamine cannot only exert its protective effects via the inhibition of hydroxyl radical production but also can inhibit PQ uptake by type II alveolar cells (Dinis-Oliveira et al., 2008; van der Wal et al., 2011). As deferoxamine is a polyamine like structure, it can compete with polyamine transporters in alveolar cells and thus inhibit the entrance of PQ in cells (van der Wal et al., 2011).

4.2. Vitamin E

Vitamin E is a lipid-soluble vitamin that serves as an antioxidant by the elimination of free radicals and stabilization of PUFAs. It has been reported that vitamin E deficiency increases acute PQ toxicity in animals, highlighting the important role of vitamin E in PQ toxicity (Dinis-Oliveira et al., 2008).

In a study by Block et al., it has been shown that vitamin E deficiency results in decreased survival and deterioration of histological lung damages in rats exposed to PQ (Block, 1979). Also, Bus et al. showed that vitamin E deficiency resulted in a significant reduction of LD₅₀ in mice, followed by contact with PQ. Although protective mechanisms of vitamin E against PQ toxicity are not well established, they may be associated with the antioxidant properties of vitamin E in the prevention of lipid peroxidation or elimination of superoxide radicals (Dinis-Oliveira et al., 2008; Fahim et al., 2013), and consequently inhibition of ferroptosis. Lipophilic antioxidants like vitamin E probably exert their inhibitory effect on ferroptosis via trapping free radicals for suppression of lipid peroxidation (Feng and Stockwell, 2018).

4.3. β -Mercaptoethanol

β -Mercaptoethanol (2-mercaptoethanol or β -ME), a thiol antioxidant with low molecular weight and has an unfavorable smell at high concentration, is used as a food additive by virtue of its reducing features. It directly interacts with some oxidized radicals and can chelate metal ions. It is assumed that β -ME acts as a scavenger of ROS

(Rafique et al., 2016; Wong et al., 2014). Some research studies have reported the health-promoting properties of long-term β -ME consumption in normal brain chemistry, longevity, weight control, tumor suppression, and immunologic function (CLICK, 2014; Wong et al., 2014). Wong et al. indicated that long-term therapy with β -ME decreases the level of systemic oxidative stress and improves the health of overweight and insulin resistance animals (Wong et al., 2014).

β -ME is considered as a ferroptosis inhibitor compound that acts through reducing the extracellular cystine to cysteine (Stockwell et al., 2017). In fact, β -ME uses cysteine as a precursor of GSH through cysteine oxidation, and its entrance to the cells stimulate GSH production. GSH is a tripeptide that has strong antioxidant activity in the elimination of ROS and maintenance of the redox cycle inside cells (Feugang et al., 2004). Recently, iron-sulfur clusters of *Paracoccus denitrificans* membrane enzymes (which are similar to our mitochondrial membrane enzymes) were found to be the subject of ROS toxicity induced by PQ, and β -ME was able to revive these enzymes activity (Sedláček and Kučera, 2019).

It can be contemplated that according to the antioxidant, ROS removal, and metal-chelating properties of β -ME, it suppresses the Fenton reaction, which has an important role in lipid peroxidation, and subsequently, ferroptosis. Therefore, based on the mechanism of PQ toxicity, the role of lipid peroxidation in this toxicity, and the relationship between lipid peroxidation and ferroptosis, β -ME can be postulated as a potential detoxifying agent in the treatment of PQ toxicity.

4.4. Ferrostatin-1

Ferrostatin-1 is considered to be a potent inhibitor of iron-induced lipid peroxidation and, therefore ferroptosis (Yangchun Xie *et al.*, 2016b). Ferrostatin-1 suppresses ferroptosis induced by lipid peroxidation, but it cannot repress the activation of apoptosis and necroptosis. There are no studies in the literature to describe the effect of this compound on the inhibition of PO toxicity.

This molecule is one of the favorite tools in laboratories for the examination of the effects of different compounds on induction or suppression of ferroptosis (Nagata and Nakano, 2017). Ferrostatin-1 is documented as a lipophilic antioxidant (Horwath et al., 2017), and although it has not yet been fully established how ferrostatin-1 inhibits

ferroptosis, the same mechanism for ROS inhibition (like previous compounds) is thought to be involved in survival of viable cells.

4.5. Edaravone

Edaravone is a medication used for the treatment of amyotrophic lateral sclerosis (ALS), and for acute-phase cerebral infarction (Watanabe et al., 2018). A very recent study revealed that edaravone is effective in protecting the lung, kidney, and liver injury induced by PQ, by lessening oxidative stress and inflammatory responses in humans. The expression of IL-6, IL-10, MDA and also TNF- α were reduced in the serum of edaravone treated patients, and the activity of superoxide dismutase (SOD) was elevated (Yi et al., 2019). It is documented that the pulmonary damage of PQ is related to elevated serum levels of IL-6 and TNF- α and a substantial elevation in the expression of NF- κ B (Dinis-Oliveira et al., 2009). Edaravone, in that study, did not improve the pulmonary fibrosis by PQ but prolonged the time required for the generation of the pulmonary fibrosis. Homma and coworkers in 2019, discovered that edaravone could protect against ferroptosis cell death *in vitro* (Homma et al., 2019). The authors described that the direct scavenging activity by edaravone is unlikely because of its slow reaction with superoxide radicals. The researchers of that study ascribed the scavenging activity of edaravone to its ability to diminish the oxidative stress in the condition of cysteine deprivation in mitochondria, but the exact mechanism was not proposed. The small structure of edaravone empowers it to readily enter many biological environments and exert its ROS and RNS scavenging activity.

4.6. MK-801 (dizocilpine)

MK-801, also known as dizocilpine, is a noncompetitive antagonist of the N-methyl-D-aspartate receptor (NMDAR), which is a type of glutamate receptors. It is well accepted that NMDAR is involved in the proliferation of fibroblasts in lung tissue (Wang et al., 2018). It is also recognized that lung fibrosis is one of the major complications of PQ toxicity (Eddleston et al., 2003). Hence, antagonizing NMDAR might decrease this toxicity and help to bring back the normal homeostasis of the cells. Also, there is evidence that PQ mediates neuronal death via the NMDA pathway (Shimizu et al., 2003; Stelmashook et al., 2016). Fortunately, there are some studies that demonstrate that the use of MK-801 mitigates acute oxidative injury in lung tissue, but excitotoxicity and not ferroptosis were reported to be involved in cell injury (Li et al., 2015; Said et al., 2000). Interestingly, NMDAR is recently implicated in the import of iron into neurons and L-glutamate is supposed to possess erastin-like effects (Reed and Pellecchia, 2012). These findings led us to introduce NMDAR antagonists as an antidote for PQ poisoning that suppress the ferroptosis through inhibiting NMDAR and glutamatergic pathways. However, NMDA antagonists like MK-801 might increase extracellular glutamate concentrations, which subsequently inhibits the activity of SLC7A11 and increases oxidative stress. Some studies have revealed that MK-801 might have antioxidant properties against free radicals (Kovacic and Somanathan, 2010). These reasons make MK-801 a unique molecule that abates different aspects of PQ toxicity.

4.7. Ebselen

Ebselen is an organoselenium ferroptosis inhibitor that also displays anti-inflammatory, antioxidant, and cytoprotective activity. Ebselen has been characterized as a potent scavenger of ROS and RNS (reactive nitrogen species) and an inhibitor of apoptosis and ferroptosis (Latunde-Dada, 2017; Ren et al., 2018). In an old study in 1987 by Cotgreave et al., it was discovered that the combination of N-acetylcysteine and ebselen is effective for the treatment of bipyridylum herbicides cytotoxicity like PQ (Cotgreave et al., 1987). However, the concept of ferroptosis was not described at the time, and we now

acknowledge that inhibition of ferroptosis, at least partly, is involved in this cytoprotectivity. Additionally, compounds like ebselen, chelerythrine, and apomorphine were considered to inhibit the activity of glutaminase (Chen and Cui, 2015). Glutaminase is responsible for the production of glutamate from glutamine and glutamate activates NMDAR, and as referred for MK-801, subsequent excitotoxicity and ferroptosis occur and lead to cell damage. Also, some studies propose that ebselen inhibits the activity of NADPH-oxidase which is a positive modulator of ferroptosis by interrupting the association of regulatory subunits (Smith et al., 2012). Furthermore, ebselen can inhibit inflammasome mediated inflammatory pathways (Selemedis et al., 2016), and as suggested earlier, a requisite for the toxicity of PQ. So, ebselen might contribute in these three distinct ways to inhibit ferroptosis and restore normal cellular oxidation-reduction cycle.

4.8. Propofol

Propofol is an FDA-approved short-acting general anesthetic that is commonly used in hospitals nowadays. There is strong evidence that supports the inhibitory activity of propofol on ROS mediated lipid-peroxidation (Hsing et al., 2011). Propofol has been documented as a potential ROS scavenging molecule which reduces the mortality of mice treated with PQ. In that study, surprisingly, thiopental (which is another general anesthetic with antioxidant properties (Tsuchiya et al., 2008)), failed to inhibit PQ toxicity (Ariyama et al., 2000). Also, another study suggests that propofol reduces the iron ion induced cell death, which is now dubbed as ferroptosis (Boland et al., 2000). It is reported that propofol reduces the induction of TNF- α pathway, which subsequently activates NADPH-oxidase, produces ROS, and finally triggers ferroptosis (Lei et al., 2013). Therefore inhibition of NADPH-oxidase once again might be the major mechanism fulfilled by propofol to suppress the PQ toxicity.

4.9. ACSL4 inhibitors

Pretreatment with rosiglitazone, a thiazolidinedione, has been documented to have a protective effect on acute lung toxicity induced by PQ (Liu et al., 2013). Peroxisome proliferator-activated receptor gamma (PPAR- γ) agonists like rosiglitazone, pioglitazone, and troglitazone stimulate the expression of nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 accrues the expression of enzymatic antioxidants like hemoxygenase-1, glutathione peroxidase, and superoxide dismutase to remove ROS (Elshazly and Soliman, 2019; Lijie et al., 2019).

However, it has been established that the anti-ferroptotic activity of thiazolidinediones is provoked almost exclusively by their ability to inhibit Acyl-CoA synthetase long-chain family member 4 (ACSL4) and not PPAR- γ activation (Angeli et al., 2017). ACSL4 is regarded as one of the crucial enzymes in long-chain fatty acid biosynthesis and esterification of fatty acids (Y. Li et al., 2019a,b). *Acs4*/ACSL4-deficient cells are immune to ferroptosis, and ACSL4 is heralded as one of the newest ferroptosis targets in which its inhibition stifles the lipid-peroxidation pathway and abrogation of ACSL4 activity is linked with resistance to ferroptosis (Xiao et al., 2019). Troglitazone is considered the most potent inhibitor of ferroptosis in the thiazolidinedione family, probably on account of its intrinsic antioxidant activity driven by chromanol moiety of its structure (which also endows tocopherols with antioxidant activity) (Doll et al., 2017).

Interestingly, Zhang et al. established that the activation of phosphatase and tensin homologue deleted on chromosome ten (PTEN) along with inhibition of TGF- β 1 by rosiglitazone are responsible for the anti-inflammatory and anti-proliferative effects observed as an antidote for PQ in a PPAR- γ dependent manner (Zhang et al., 2019). We also know that rosiglitazone significantly inhibits the ferroptosis death of the cells. Also, many other compounds introduced as PQ antidotes in this study were actually an inhibitor of ferroptosis. Putting the pieces of this puzzle together, we conclude that as observed by previous studies,

rosiglitazone prevents the toxicity induced by PQ and inhibition of ferroptosis is involved in this protective effect. Hence, it is rational to state that thiazolidinedione can ameliorate the toxicity induced by PQ, mainly by inhibiting ACSL4 activity, partially by boosting ROS scavenging enzymatic activity, and eventually suppressing ferroptosis.

4.10. Lipoxygenase/cyclooxygenase inhibitors

Benavides et al. reported that when sunflower leaf discs were treated with PQ an array of cellular changes were observed. Increased production of oxidative stress markers, decreased level of natural polyamines, declined activity of arginine decarboxylase (ADC) and ornithine decarboxylase (ODC) in addition to a 25% increased lipoxygenase activity were documented (Benavides et al., 2000). Also, other studies confirmed the escalation of LOX activity and the diminution of water-soluble antioxidants in plants treated with PQ (Hasanuzzaman et al., 2018).

Two recent studies demonstrated a protective role for two PUFA metabolites by activating the Nrf2 pathway. Resolvin D1 (RvD1) is produced as a metabolite of docosahexaenoic acid (DHA) by LOX and is successfully applied to halt inflammation in animal models. In the presence of aspirin, the acetylated form of cyclooxygenase-2 (COX-2) generates aspirin triggered-resolvin D1 (AT-RvD1), which has a more potent anti-inflammatory activity and stability. AT-RvD1 prevented PQ-induced oxidative stress and acute renal injury by mobilizing Nrf2 pathway and downstream anti-oxidative related genes in mice (X. Hu et al., 2019a). Also, AT-RvD1 effectively suppressed the PQ-induced inflammation in the lung and pulmonary edema in mice (X. Hu et al., 2019b).

Another study on the mechanistic investigation of PQ toxicity asserted that PQ induces the formation of leukotriene B₄ in rat lungs, which is supposed to be mediated by the increased 5-lipoxygenase (5-LOX) activity (Hoffer et al., 1997). GSH depletion, which is associated with PQ toxicity, activates 12-lipoxygenase (12-LOX), culminating in the production of peroxides and cell death (Nellore and Nandita, 2015). Although up to now, it is roughly accepted that LOXs are not prerequisites for the execution of ferroptosis, their contribution in the inception of cellular pathways implicated in the generation of lipid hydroperoxides that trigger ferroptosis is indubitable, and pan-LOXs inhibitors might be effective candidates for impeding ferroptosis (Shah et al., 2018).

On the other side, 5-LOX inhibitor zileuton granted HT22 (mouse neuronal cell line) protection against glutamate-induced oxidative injury by quenching ferroptosis (Liu et al., 2015). Baicalein and nordihydroguaiaretic acid (NDGA), inhibitors of human lipoxygenases, protected cells from RSL3-stimulated lipid peroxidation, ROS production, and cell death, indicating that LOXs play an important role in regulating ferroptosis (Probst et al., 2017). It is well acknowledged that flavonoids like quercetin, inhibit the activity of 15-LOX, and by this means (along with other possible antioxidant mechanisms) may lessen the oxidative damage induced by PQ (Park et al., 2010; Sadik et al., 2003; Zerin et al., 2013).

Some studies enunciated a selective increase in the production of prostaglandin F₂ alpha (PGF₂α) in the lung for PQ, prior to its pulmonary edema formation. Ibuprofen, a widely used NSAID, effectively blocked these two complications of PQ toxicity (i.e. edema and increased PGF₂α) (Lindenschmidt et al., 1983). Although COXs, which are antagonized by NSAIDs, are not thought to have a direct effect on ferroptosis, they prepare the groundwork for inflammatory modulators, which amplify ROS and oxidative stress to the cells. Some NSAIDs were also found to possess iron-chelating activity and antioxidant properties (Kovacic and Edwards, 2011). NSAIDs that display these features like hydroxamic acid derivatives (ibuprofen, fenoprofen, diclofenac, etc. (Končić et al., 2009)) but not acetaminophen (which bankrupts GSH reservoir of the cells) seems to be interesting choices for the treatment of PQ poisoning.

There are some other intriguing findings that emphasize the role of arachidonic acid metabolism (LOX and COX) in PQ toxicity and ferroptosis. Angiotensin II initiates the signaling cascade of ERK/MAPK/NF-κB/ROS, which in turn stimulates COX-2 activity. Losartan, an angiotensin receptor blocker (ARB), was reported to have a remarkable protective role in lung tissue after PQ toxicity (lower inflammatory markers such as TGF-β1, matrix metalloproteinase 9 (Mmp9), tissue inhibitor of metalloproteinase 1 (TIMP-1), collagen and reduced pulmonary fibrosis) (Guo et al., 2015). Surprisingly, it has been discovered that angiotensin II affects the expression of many proteins involved in iron homeostasis (TfR1, DMT1, Fpn1, hepcidin, etc.), which ultimately increases the intracellular level of iron (Huang et al., 2014). It can be speculated that losartan at least to some degree, confiscates the requirements for induction of ferroptosis, by preventing COX-2 and ROS formation and reducing the accumulation of iron inside the cells. Moreover, captopril, an inhibitor of angiotensin-converting enzyme (ACE), which prevents the conversion of angiotensin I to angiotensin II, also attenuates the toxicity induced by PQ in rat liver (Ghazi-Khansari and Mohammadi-Bardbori, 2007). In that study, the protective role of captopril was ascribed to its antioxidant properties due to its thiol group. Lower activation of the angiotensin pathway may be another important or even the main protagonist of this detoxification mechanism instead of the simple “thiol-antioxidant” theory. In another study, enalapril and captopril failed to reduce the lipid peroxidation and increase GSH levels but did reduce the fibrosis associated with PQ (Ghazi-Khansari et al., 2007). Lipid peroxidation is more closely linked with the intrinsic antioxidant capability of molecules, and in this manner, captopril is a weak antioxidant and enalapril which lacks the thiol group compared with its congener, is completely devoid of intrinsic antioxidant activity. Therefore, paradoxical results were found but fibrosis, which is the ultimate complication of ferroptosis (Gong et al., 2019), especially in the case of PQ, is hampered by both ARBs and ACEIs and angiotensin II signaling pathway, and this mechanism is the commonality observed for both of these agents. The structures of possible antidotes for PQ poisoning which act by regulation of ferroptosis are illustrated in Fig. 4.

5. Statins and PQ toxicity, a complex paradigm

In a study conducted by Khodayar et al., authors investigated the effect of atorvastatin administration on PQ toxicity, and the results uncovered that atorvastatin could alleviate the pulmonary fibrosis and inflammation in PQ-treated rats (Khodayar et al., 2014). Potent free radical scavenging activity and antioxidant characteristics were proposed for atorvastatin in that study, and the authors linked the protective effect of atorvastatin to PQ-toxicity by this means. Also, some other investigations revealed that atorvastatin could protect neurons against oxidative stress by activating PI3K, ERK, and free radical scavenging activity. Moreover, diminution in the hydroxyproline and malondialdehyde levels, induced by paraquat, was traced by treating with atorvastatin (Lee et al., 2016). Simvastatin, another famous statin, failed to supply a beneficial effect on the PQ-induced stress on *Drosophila melanogaster* (Spindler et al., 2012).

Statins are best-known for their pleiotropic effects, meaning that their beneficial effects cannot be solely attributed to the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. In other words, statins can improve the function of vascular endothelial cells, diminish oxidative stress and inflammation, protect cardiac cells, slow down the progression of renal damage, and finally reduce platelet activation and aggregation (Diamantis et al., 2017). Previous studies demonstrated both pro-apoptotic and anti-apoptotic effects for statins, and there is no unanimous agreement about the way in which these drugs are related to cell death and survival (Wood et al., 2013).

Hao and colleagues in their recent review article maintained the idea that although there is no consensus view about the correlation of statins and ferroptosis, statins display a slight edge toward promoting

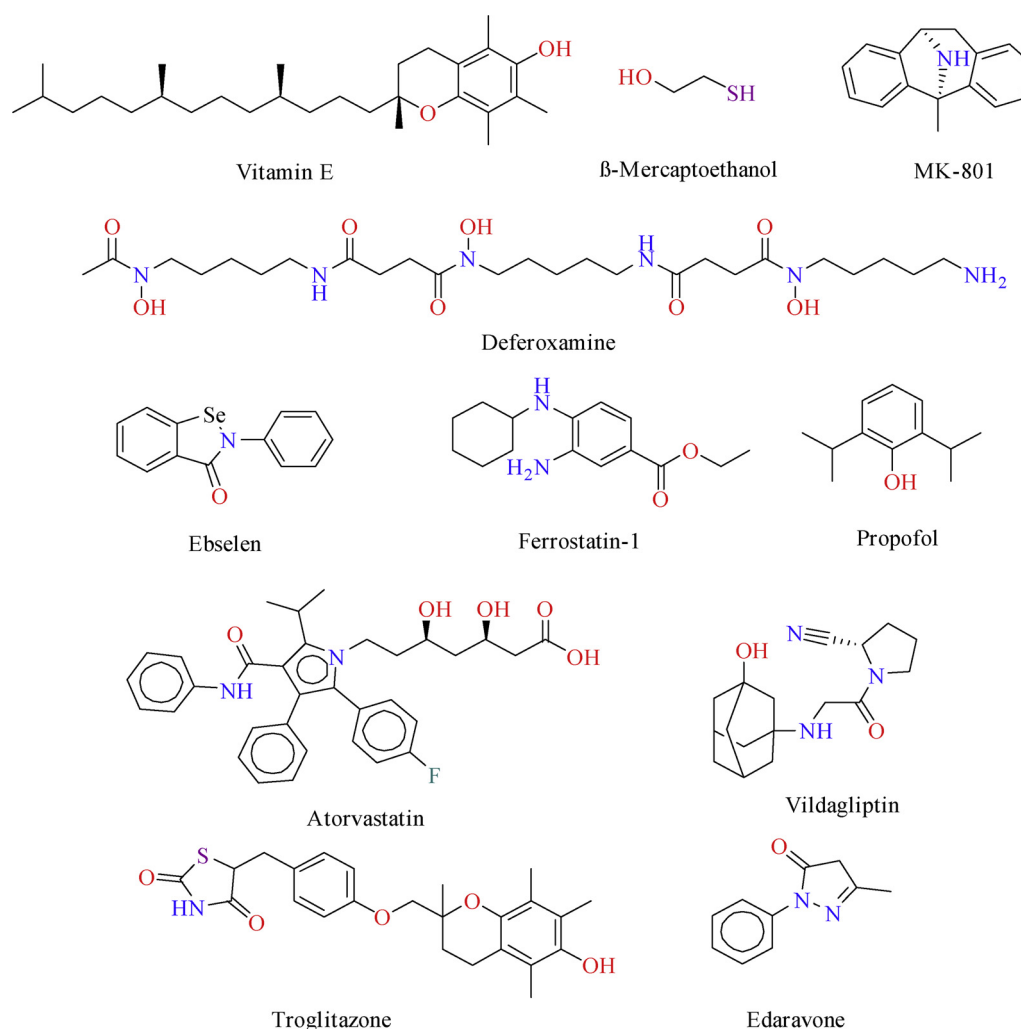


Fig. 4. An overview of medications interfering with ferroptosis which can be utilized for the treatment of PQ toxicity.

ferroptosis by down-regulating the mevalonate pathway (a direct result of HMG-CoA reductase inhibition), which is a critical signaling element for GPX4 maturation (Hao et al., 2018). An elevated level of sensitivity to ferroptosis for some cancer cell lines, presumably caused by hampering the synthetic pathway of GPX4 is another observation for a different statin, fluvastatin. This treatment resulted in an augmentation of cellular lipid peroxidation, which is an aftereffect of GPX4-reduced gene expression (Viswanathan et al., 2017). Depletion of CoQ (a product of the mevalonate synthetic pathway) reservoirs, a crucial membrane antioxidant, is another observation for the cells treated with statins, which uphold the ferroptosis pathway even further (Dixon and Stockwell, 2019).

In this regard, statins seem to subvert their other positive anti-inflammatory and free radical scavenging effects. Thus, the results of the discussed studies indicate that when the radical scavenging and anti-inflammatory activities of statins overwhelm their pro-ferroptotic (and anti-GPX4) activity, statins behave like an antidote for PQ and when the pro-ferroptotic activity is stronger, no beneficial or a toxic effect might be the case.

6. Crosstalk of dipeptidyl-peptidase-4 (DPP-4) and ferroptosis

A recent study in Cell Reports established that the tumor suppressor protein p53 (TP53), hinders the process of ferroptosis by preventing the activity of dipeptidyl-peptidase-4 (DPP-4) in a transcription-independent way (Xie et al., 2017). DPP-4 is essentially a peptidase

enzyme, capable of cleaving many biological peptides, but according to the mentioned study, the enzymatic function of DPP-4 seems unrelated to ferroptosis. DPP-4 non-enzymatic activities like binding to NADPH oxidase 1 (NOX1) appears to be required for lipid-peroxidation in ferroptosis in only TP53-deficient cells. Indeed, TP53 localizes DPP-4 from the membrane to its inactive nuclear form in CRC cell lines and therefore gliptin drugs like sitagliptin, saxagliptin, linagliptin, and vildagliptin, which inhibit the activity of this key enzyme, are potential therapeutic compounds and can be used for the prevention of ferroptosis (Lei et al., 2019; Xie et al., 2017).

Interestingly, distinct but relatively recent surveys on different DPP-4 inhibitors have demonstrated that they lower the level of ROS and improve mitochondrial functionality. Pretreatment of human umbilical vein endothelial cells, which were incubated with high glucose, with sitagliptin significantly reduced the ROS levels, prevented mitochondrial membrane potential collapse and suppressed apoptosis (Wu et al., 2017). Saxagliptin treatment stopped podocyte epithelial-to-mesenchymal transition (EMT) by inhibiting the activity of stromal cell-derived factor-1 α (SDF-1 α), and prevention of subsequent oxidative stress (Chang et al., 2017). Another study on saxagliptin revealed that this drug reduces ROS and increases the level of GSH in chondrocytes presumably via p38/I κ B α /NF- κ B pathway (N. Hu et al., 2019). Vildagliptin could decrease brain mitochondrial swelling, brain mitochondrial ROS levels, and mitochondrial membrane potential depolarization and by this means ameliorates brain mitochondrial dysfunction (Zhang et al., 2018). Linagliptin, alogliptin, and vildagliptin were found to decrease

the sensitivity of cells to ferroptosis by diminishing DPP-induced production of lipid peroxides (Stockwell et al., 2017). In summary, DPP-4 inhibitors are recent and novel agents that can delay the progression of ferroptosis in cells, and this mechanism could provide some insights into newer antidotes for PQ toxicity.

7. VDAC modulators: promising future?!

Voltage-dependent anionic channels (VDACs) are porin like proteins that regulate mitochondrial ionic, metabolic and energetic homeostasis. Small hydrophilic molecules can pass through these channels (De Pinto et al., 2010). It is also documented that NADH adjusts VDAC activity. VDAC1 is considered as one of the mediators of PQ toxicity, possibly via an NADH dependent manner (Shimada et al., 2009). VDAC2 and VDAC3 (possibly directly) are regarded as the target proteins in erastin-induced cell death, and it is believed that this binding of erastin opens up the VDAC channel and leads to increased mitochondrial ROS generation, which impairs the normal reduction-oxidation cycle, and finally promotes ferroptosis (DeHart et al., 2018; Yu et al., 2017). Owing to the cationic nature of amines in PQ structure, this agent might bind directly to these receptors and alter their regular activity (Shimada et al., 2009). Modulators which bring back the normal function of these channels are one of the nominees for newer agents of antidotes to treat PQ poisoning. According to the drugbank database, until now, there is no experimental, investigational, or approved drug or chemical to target VDAC proteins with definite pharmacological activity. Thus, future researches are urgently needed to develop our understanding of these important protein channels and ligand molecules.

The findings of this study are summarized in Table 1. Note that the amount of previous literature data supporting the type of compounds that inhibit the PQ-induced toxicity is not straightforwardly proportional to the amount of toxicity they can suppress and their beneficial effect. For instance, DPP-4 inhibitors, ACSL4 inhibitors, and statins display a variety of protective effects and are very promising, but there is a dearth of evidence to back up their usefulness.

8. Concluding remarks

Considering that paraquat (PQ) poisoning is widespread and difficult to manage, providing a robust approach for the treatment of intoxicated patients is highly encouraged. Insufficient understanding of

PQ poisoning and obfuscations in the treatment mechanisms of its antidotes which were only partially effective in the past motivated us to survey the intersection of ferroptosis, a recently defined form of cell death, with PQ poisoning and the compounds which have been used for management of PQ toxicity via this mechanism. Many of these treatment protocols are exploiting ferroptosis inhibition to overcome PQ toxicity, but only the observation of cytoprotectivity for many of these compounds have been characterized in previous works. By reviewing the literature, we can rationally propose ferroptosis inhibitors as useful agents for the treatment of PQ poisoning.

Our current knowledge supports the fact that lipid peroxidation and PQ toxicity coexist with each other, and cell death in many cases was observed in which the exact physiopathological mechanism underlying them is not yet elucidated. Some sporadic reports have also reported that ferroptosis can be the result of PQ toxicity.

Although deferoxamine was used as a therapeutic drug for the management of PQ toxicity, the interconnection between these the biological pathways of these two compounds has not yet been fully characterized, and it was suggested that the chelating activity of deferoxamine is not the main interplay (Burkitt et al., 1993). Ferroptosis might be the bridge in which deferoxamine antidote activity is exerted.

Similarly, the irrefutable relationship between vitamin E and prevention of ferroptosis (Hinman et al., 2018) as well as the observation that vitamin E deficiency is linked to higher sensitivity to PQ poisoning (Block, 1979) led us to propose this antioxidant for management of PQ intoxication.

β -ME suggested in this study has long been used for the treatment of methylmercury (MeHg) poisoning (Thayer, 1974), and it is clearly acknowledged that metal toxicity at least partly is mediated by generation of ROS (Farina et al., 2013). The cellular overload of oxidative stress, as mentioned in the text, is the driving force for ferroptosis, and the reason that β -ME has been used for the treatment of methylmercury poisoning might be attributed to this inhibitory effect of β -ME on ferroptosis. As emerging studies related to the ferroptosis mediated toxicity of PQ are being investigated, the role of some other specific ferroptosis inhibitors like ferrostatin-1 would be more embracing. Edaravone, another small lipophilic antioxidant, displays a bright perspective as an inhibitor of ferroptosis to slow-down and decrease the PQ toxicity.

NMDAR is another attractive target, and by reviewing the literature, ferroptosis and not excitotoxicity alone appeared to be the key in PQ poisoning. NMDAR antagonists like MK-801 would be interesting

Table 1
The intersection of ferroptosis and paraquat-induced toxicity.

Compounds or its class	Main Indications or Usage	Mechanism of inhibition of ferroptosis	Evidence for inhibition of PQ toxicity
Deferoxamine	Iron poisoning	Trapping iron	(Kohen and Chevion, 1985; Van Asbeck et al., 1989; van der Wal et al., 2011)
Vitamin E	Vitamin Supplement	Suppressing ROS and lipid peroxidation	(Block, 1979; Davarpanah et al., 2015; Fahim et al., 2013; Shahar et al., 1980; Watanabe et al., 1986)
β -Mercaptoethanol	Food additive, metal poisoning	Increasing GSH levels, antioxidant activity	(Sedláček and Kučera, 2019)
Ferrostatin-1	Laboratory assessment of ferroptosis	Lipophilic antioxidant and ROS scavenger	–
Edaravone	ALS, cerebral stroke	Mitochondrial ROS and RNS scavenger	(Homma et al., 2019; Shokrzadeh et al., 2014; Zhi et al., 2011)
Dizocilpine (MK-801)	Recreation	NMDAR antagonism (ceasing the entrance of iron into cells)	(Said et al., 2000; Shimizu et al., 2003)
Ebselen	Research for diseases with ROS basis	Strong scavenger of ROS and RNS, Inhibition of glutaminase, suppressing the inflammasome pathway	(Cotgreave et al., 1987)
Propofol	Anesthesia	Inhibition of NADPH-oxidase, Antioxidant and ROS scavenging activity	(Ariyama et al., 2000; Del Valle Lugo-Vallin et al., 2002; Shi et al., 2006)
ACSL4 inhibitors	None to date	Inhibition of fatty acids biosynthesis and their subsequent oxidation	(Liu et al., 2013)
LOX/COX inhibitors*	Anti-inflammatory and analgesic	Inhibition of oxygenase-mediated ROS, iron chelation (some NSAIDs)	(X. Hu et al., 2019a,b; Lindenschmidt et al., 1983; Zerin et al., 2013)
Statins	Cholesterol-lowering	Promoting ferroptosis!	(Khodayar et al., 2014)
DPP-4 inhibitors	Diabetes mellitus	Inhibition of NADPH-oxidase recruitment, decreasing ROS and improving mitochondrial membrane functionality	–
VDAC modulators	None to date	Preventing mitochondrial ROS generation	(Shimada et al., 2009)

especially when they are co-administered with another potent lipophilic antioxidant like vitamin E. Ebselen, a synthetic selenium-containing organic compound, by its intrinsic antioxidant properties as well as NMDAR and NADPH-oxidase inhibitory activity, contribute to reducing the toxicity of PQ via ferroptosis and ROS to the cells.

Thiazolidinediones inhibit the activity of ACSL4, and ACSL4 double knockout mice are impervious to ferroptosis. Although the behavior of ACSL4 is rather complicated and its inhibition is not linearly correlated with ferroptosis sensitivity, researchers have found an “all-or-nothing” role for this protein in ferroptosis, meaning that after a certain threshold there is no increase in the sensitivity of cells to ferroptosis (Doll et al., 2017). More research is needed to disclose the intersection between ACSL4 and ferroptosis with PQ-induced toxicity.

The interrelationship between cyclooxygenase/lipoxygenase pathways and ferroptosis is rather oblique. Lipoxygenase inhibitors seem to be more auspicious in this regard, and NSAIDs with inherent antioxidant and (iron-) chelating features are other candidates for the cessation of ferroptosis in susceptible cells exposed to PQ.

Pleiotropic nature of statins has made them a potential candidate for preventing ferroptosis by radical scavenging and anti-inflammatory activity, but they are also capable of reducing the expression of GPX4, and by this means, display a dual nature.

Gliptins are being considered as anti-inflammatory compounds with versatile cytoprotective properties. They decrease the ROS levels and improve mitochondrial membrane functionality, but no study has been explored the role of these newer chemicals in the treatment of paraquat toxicity to date. Gliptins, statins, and VDAC modulators might display more beneficial effects when they would be combined, which could confer them a synergistic or additive activity on PQ-intoxicated victims.

VDAC proteins are increasingly known for their role in ROS mediated cell deaths (apoptosis along with ferroptosis) (Lemasters, 2017). There is no research focusing on the role of VDAC modulators for the treatment of PQ toxicity, mainly due to our inefficient understanding of the interaction of chemicals with these channels. Untangling the definite physiological role of VDAC in cell viability and striving for newer medications targeting these proteins might shed light on better PQ treatment approaches.

Declaration of Competing Interest

The authors declare no conflict of interest.

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