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Review article

# FERROPTOSIS AND ITS POTENTIAL ROLE IN CARDIOVASCULAR DISEASES

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**ABSTRACT**

Iron (Fe) is the fourth most common element in the earth's crust, and plays a significant role in the human body.It is important for cell survival due toits involvement in the transport of oxygen, in DNA biosynthesis, adenosine triphosphate (ATP) synthesis, as well as an auxiliary factor of various proteins in the tricarboxylic acid cycle (TCA). It has also been found that iron is closely related to the onset and progression of various cancers and disturbances in its metabolism can stimulate the growth of tumor cells. In addition, divalent iron significantly accelerates the lipid peroxidation of saturated fatty acids.

**Keywords:** Ferroptosis, adenosine triphosphate (ATP), erastin, cell death, apoptosis, necrosis,

## INTRODUCTION

During the oxidative phosphorylation in mitochondria, which is an iron-dependent process, along with the production of ATP, reactive oxygen species (ROS) are also generated. The generated in large quantities oxygen radicals, exceeding the capacity of the reductase mechanisms of the cells, can result in direct or indirect injury to different molecules such as proteins, nucleic acids and lipids [1], and consequently also to cell death. This newly discovered form of cell death is called ferroptosis. It differs from apoptosis and necrosis and is a result of the accumulation of iron-dependent lipid peroxidase [2].

Typical morphological changes are shrinking of cell volume and increase in the density of the mitochondrial membrane [2]. Ferroptotic cell death is induced by two different classes of small molecules. Class I inducers of ferroptosis include erastin, sulfasalazine (SAS), dihydroxyphenyl- imino-2-imidazolidine (DPI2) and buthionine sulfoximine, which reduce the intracellular glutathione level, causing an imbalance between oxidation-reduction in the cells. Class 2 ferroptosis inducers include Ras selective lethal compound (RSL3), DPI7, DPI10, DPI12, DPI13, etc. that directly inhibit glutathione peroxidase 4 (GPx4) [3] and eventually lead to accumulation of lipid peroxides in the cell. In addition, ferroptosis can be induced by various drugs (e.g., sorafenib, artemisinin and its derivatives) [4, 5].

Regulators of ferroptosis are mainly the mevalonate pathway, the sulfur transfer signaling pathways and the heat shock protein 1- heat shock factor 1 (HSF1-HSPB1) system [6]. This type of cell death can occur during various physiological and pathological processes in humans and animals. The research describes the involvement of ferroptosis in various disorders in humans. In particular, by regulating the growth and proliferation of tumor cells, ferroptosis plays an important role in the development and progression of various cancers. Up until now susceptibility of tumor cells to ferroptosis has been reported for pancreatic ductal carcinoma, renal cell carcinoma (RCC) and hepatocellular carcinoma (HCC) [3, 7, 8].

### Discovery of ferroptosis

Historically, the inducers of ferroptosis were found initially, before the process was described. In a 2003 study using large-scale screening to investigate the cytolytic effect of various chemical compounds on tumor cells, Stockwell et al. identified a new chemical compound, called erastin. This compound has the ability to induce death in tumor cells with a RAS mutation, in a manner different from apoptosis [9]. In 2008, Stockwell et al. discover two new molecules - RSL3 and RSL5, which have the same effect as erastin. In this study, it was found that the observed cell death can be inhibited by the iron chelator - desferrioxamine B-methanesulfonate (DFOM) and the antioxidant vitamin E [10], thus confirming that this form of cell death is associated with the intracellular ferrous ion and the reactive oxygen species. In 2012, Stockwell et al. used the term "ferroptosis" to describe this type of cell death, caused by the accumulation of iron-dependent lipid peroxides [2]. Later, other compounds have been determined as well (sorafenib [11], artemisinins [5,7], cyclic peroxide 1,2-dioxolane - FINO2) [12], that have the ability to induce ferroptosis. In addition, the activation mechanisms for ferroptosis molecular systems Xc- (cystine-glutamate antiporter that is chloride dependent) and GPx4 [3,13] have been discovered. Recently, a relatively detailed summary of ferroptosis inducers, inhibitors and regulatory molecules has been published [14].

**Differences between ferroptosis and apoptosis / necrosis**

Cell death, which represents the end-stage of the cell cycle, is caused by various cytotoxic exogenous or endogenous substances. In 1972, Kerr et al. described "automatically programmed" cell death, which they called apoptosis [15].

Later, by using electron microscopy, Schweichel and Merker observed types of cell death of embryonic cells in rats that were treated or not with lethal to the embryo substances. They divided the cell death into three types [16].

Clark named the third type – necrosis [17], which is a passive form of cell death. With the advance of scientific research in this area, various types of cell death, such as pyroptosis, necroptosis, parthanatos, autophagy, oncosis and ferroptosis were discovered. The latter form differs significantly from other types such as apoptosis, necrosis and autophagy including by its morphological, biochemical and genetic features [2,10].

Ferroptosis does not lead to morphological changes, such as the condensation of the chromatin that occurs during apoptosis, loss of the plasma membrane integrity of the cell that occurs during necrosis or the formation of autophagic vacuoles that appear during autophagy. Instead, ferroptosis is characterized primarily by a reduction in the volume of mitochondria and an increase in density with subsequent rupture of the external mitochondrial membrane [2].

The induction of ferroptosis is done by different types of low molecular weight molecules such as erastin and RSL3. It is interesting to note that it cannot be induced by substances that are triggers for apoptosis and necrosis[5], suggesting that the mechanism underlying ferroptosis differs from that of apoptosis and necrosis. In addition, the inducers of ferroptosis generally differ from those of other newly discovered forms of cell death, such as necroptosis and pyroptosis [18].

A recent study has found a link between ferroptosis and autophagy, which share some common mechanisms [19]. Therefore, the relationship between ferroptosis and other types of cell death remains to be elucidated.

The cysteine / glutamate transport system Xc- is a membrane Na+ -dependent cysteine-glutamate exchange transporter, which is a disulfide–linked heterodimer consisting of a light (xCT, SLC7A11) and heavy chain (CD98hc, SLC3A2) [20]. It transports extracellular cystine inside the cell [21], which is then transformed into cysteine used for glutathione synthesis (GSH). This molecule is critical for the protection of the cell against injury caused by oxidative stress [22].

In the central nervous system (CNS), the neurotoxicity of glutamate is oxidative iron-dependent [23, 24]. It can be inhibited by iron chelators and ferr-1 [2], which means that an involvement in ferroptosis is possible [23]. Previous studies have revealed that glutamate toxicity is due to an increased calcium influx in the cell or is a result of inhibition of the Xc- system by competitive inhibitors [21, 25]. However, Wolpaw et al. reported that calcium chelators did not affect ferroptosis [26]. The latter conclusion supports the thesis that the activation of glutamate receptors is not involved in ferroptosis [18].

**Ferroptosis and cardiovascular system**

Despite the role that ferroptosis plays in the pathogenesis of various oncological diseases is being studied extensively, the role of ferroptosis in cardiovascular disease is not so well researched.

Initially, ferroptosis has been observed in cancer cells expressing an oncogenic Ras gene, and subsequently in other disorders such as Huntington's disease and renal failure [27], but there are few studies on the role of the process in cardiovascular disease. Nevertheless, the study of iron homeostasis and the involvement of iron in the myocardial damage has a long history. The term "iron overload cardiomyopathy" has been introduced to designate a type of myocardial damage, resulting from the accumulation of iron in the myocardium, primarily due to genetically determined disorders of iron metabolism or as a result of multiple blood transfusions [28].

The clinical use of doxorubicin is limited due to its cardiotoxicity. In a study, the concomitant use of iron chelators together with doxorubicin resulted in limiting the cardiotoxicity, which raises the question of the likely involvement of iron in myocardial injury, mediated by the chemotherapeutic agent [29]. Nitenberg et al. showed that in patients with diabetes mellitus and heart failure (HF), the iron homeostasis is possibly impaired, and chelation therapy may improve coronary microvasculature [30].

In a recent study by Lapenna et al. [31] have been reported higher levels of low molecular weight iron (LMWI) - a redox-active catalytic form of iron, as well as higher levels of lipid and protein oxidation in the hearts of adult rabbits as compared to the younger controls. These results show that ferroptosis, characterized by an increased oxidative stress, is probably one of the major mechanisms of cell death leading to cardiac dysfunction. However, the iron homeostasis and associated with it oxidative stress are still not enough studied in heart failure caused by increased preload. Therefore, future research into potentially effective therapeutic strategies, aimed at influencing ferroptosis, is needed.

As a tissue that consumes a large amount of oxygen, the heart is rich in unsaturated fatty acids, which provides the pathophysiological basis for the generation of reactive oxygen species with subsequent myocardial damage [32].

In addition, in situations of stress, mitochondrial or functional impairment, the abundance of non-heme-bound iron in the myocardium can cause lipid peroxidation of the cell membrane through the Fenton reaction (reaction between hydrogen peroxide and ferrous ions) [32].

There are a number of studies that demonstrate the key role of oxidative stress in cardiomyocyte death. In addition, in some cardiovascular diseases such as myocardial infarction, in physiological processes such as aging has been described regional accumulation of iron in the myocardium [28-31]. Interestingly, iron chelators and antioxidant agents can effectively reduce the death of cardiomyocytes [28-31], suggesting that ferroptosis is a possible mechanism for cell death in cardiovascular diseases.

In animal models of myocardial ischemia-reperfusion injury, the blocking of the key molecule for ferroptosis - glutamine results in a reduction in myocardial injury [33]. In another interesting study, the role of ferroptosis in the induction of ischemia-reperfusion injury has been reported [34]. Initially, the iron load and the fibrosis were assessed in cardiomyocyte populations in mice, subjected to an ischemia-reperfusion injury. It is significant to mention the accumulation of iron in cardiomyocytes and the development of excessive fibrosis around the deposits, one week afterischemia-reperfusion injury (I / R injury). In order to assess the degree and presence of ferroptosis in these cardiomyocytes, the authors use separately three molecules (erastin, RSL3, and Fe3+), leading either to an increase in cellular iron levels or to increased iron-mediated oxidative stress. As a result, increased intracellular production of ROS and excessive cell death were recorded. The so observed changes were suppressed by the administration of a ferroptosis inhibitor - ferrostatin-1. In the same study, in cardiomyocytes with excessive iron accumulation, no molecular changes typical of autophagy or apoptosis were detected [34].

Authors also [34] report the protective role of cardiomyocytes of the mammalian target of rapamycin (mTOR) - a key effector in the insulin signaling pathway, which regulates not only cellular metabolism but also cell survival against excess iron and ferroptosis.

In another study was reported cell death with the morphological features of ferroptosis [32]. The experiment was conducted in rats with heart failure that was experiment tally induced by ligation of the thoracic aorta and in the H9c2 myocytes (a subclone of an original clonal cell line, derived from embryonic rat heart tissue) in vitro, treated with erastin or isoproterenol (ISO). The described cell death is characterized by reduced vitality of the cell, increased lipid peroxidation and unstable iron deposits. Both rats and H9c2 cells were treated with puerarin – a phytoestrogen with antioxidant properties, approved for the treatment of cardiovascular and other diseases in China [35]. Interestingly, lipid peroxidation and iron overload are significantly blocked, following the administration of puerarin (phytoestrogen with antioxidant properties) [32]. These results provide convincing evidence that puerarin reduces myocyte loss during HF, partly by reducing ferroptosis, suggesting a new mechanism for potential therapy for heart failure.

## CONCLUSION

Despite the available studies in this area, there is now no definitive evidence of the involvement of iron-mediated ferroptosis in the pathogenesis of various cardiovascular diseases. The confirmation of this role is likely to encourage and improve the search for new therapeutic options.

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