ROS

RESEARCH HIGHLIGHTS

GPx4 in Bacterial Infection and Polymicrobial Sepsis: Involvement of Ferroptosis and Pyroptosis

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ABSTRACT | While it is well known that bacterial infection is the predominant cause of sepsis, the molecular pathophysiology of this clinical syndrome remains ill-defined. In this Research Highlights article, we discuss the recent research findings regarding a protective role for glutathione peroxidase-4 (GPx4) in bacterial infection and polymicrobial sepsis via modulating ferroptosis and pyroptosis, two novel modes of regulated cell death. It is suggested that GPx4, being a requisite gateway to both ferroptosis and pyroptosis, may serve as a critical molecular target for developing effective drugs for controlling infection and sepsis.

KEYWORDS | Bacterial infection; Ferroptosis; GPx4; Lipid peroxidation; Pyroptosis; Sepsis

ABBREVIATIONS | AA-PE, arachidonic acid-phosphatidylethanolamines; D3T, 3*H*-1,2-dithiole-3-thione; GPx, glutathione peroxidase; GPx4, glutathione peroxidase-4; GSH, reduced form of glutathione; LOH, alcohol; LOOH, organic hydroperoxide; PHGPx, phospholipid hydroperoxide glutathione peroxidase; PLCG1, phospholipase C gamma 1; ROS/RNS, reactive oxygen and nitrogen species

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1. OVERVIEW

Sepsis is the culmination of complex interactions between the infecting microorganisms and the host immune, inflammatory, and coagulation responses, leading to death due to multiorgan failure [1–5]. Substantial evidence suggests an important role for reactive oxygen and nitrogen species (ROS/RNS) and oxidative stress in the initiation and progression of multiorgan dysfunction and injury in sepsis in both experimental animals and human subjects [6]. However, exogenous antioxidant-based therapies have been unsuccessful possibly due to their limited ability to scavenge ROS/RNS and counteract oxidative/inflammatory stress. Thus, efforts have recently focused on harnessing the catalytic power of endogenous antioxidant enzymes. In this context, glutathione peroxidase-4 (GPx4), one of the GPx enzyme family, has been emerging as a novel player in counteracting bacterial infection and combating sepsis development in experimental models. In this Research Highlight article, we discuss the recent research studies leading to the discovery of a novel function of GPx4 in protecting against bacterial infection via inhibiting ferroptosis and in ameliorating polymicrobial sepsis through suppressing pyroptosis in animal models. To lay a basis for the subsequent discussion, we begin with a brief overview of the GPx enzyme family.

2. THE GPX ENZYME FAMILY

Glutathione peroxidase (GPx) is the general term for a family of multiple isozymes that catalyze decomposition of hydrogen peroxide or organic hydroperoxides using the reduced form of glutathione (GSH) as an electron donor [7]. In mammals, there are currently eight GPx isozymes, namely, GPx1, GPx2, GPx3, GPx4, GPx5, GPx6, GPx7, and GPx8. GPx1–4 isozymes are selenoproteins. GPx6 is also a selenoprotein in humans and pigs, but not in rats and mice. GPx5 is a non-selenoprotein. GPx7 and GPx8 are the newest members of the family and have been shown to be endoplasmic reticulum-resident protein disulfide isomerase peroxidases [8].

GPx1–6 isozymes are all able to catalyze the reduction of H_2O_2 or organic hydroperoxides (LOOH) to water or corresponding alcohols (LOH) (**Figure 1**). GPx4 is also able to reduce phospholipid hydroperoxides in cell membranes, and as such, is called phospholipid hydroperoxide GPx (PHGPx). Due to the low levels of GSH in extracellular fluid, GPx3 (also known as plasma or extracellular GPx) may also use extracellular thioredoxin and glutaredoxin as electron donors [9]. In addition to decomposing H_2O_2 and LOOH, GPx isozymes can reduce peroxynitrite in vitro [10]. The exact biochemical functions of the newly discovered GPx7 and GPx8 remain ill-defined though evidence suggests a role in maintaining cellular redox homeostasis [11, 12].

3. GPX4 IN FERROPTOSIS AND BACTERIAL INFECTION

3.1. GPx4 and Ferroptosis

In humans, GPx4 gene is localized on chromosome 19p13.3. As noted earlier, GPx4 is also known as PHGPx. This 20–22 kDa monomeric enzyme is ubiquitously expressed in a variety of tissues. It is also found to be a main structural component of the sperm mitochondrial capsule in mature spermatozoa, where it exists as an enzymatically-inactive, oxidatively cross-linked, insoluble protein [13]. Subcellular distribution of GPx4 includes the cytosol, nuclei, mitochondria, and membranes.

Membrane-associated GPx4 plays an important role in repairing oxidative damage to membrane lipids. Recently, GPx4 is found to be an inhibitor of ferroptotic cell death via selenium utilization [14, 15]. Ferroptosis is a newly discovered type of cell death that differs from apoptosis, necrosis, and autophagy, and results from iron-dependent lipid peroxide accumulation triggered by insufficiency of GPx4 [16–18]. Inactivation of GPx4 causes ferroptosis and triggers lipid peroxidation-induced acute renal failure [19]. Conditional ablation of the ferroptosis inhibitor GPx4 in neurons results in rapid motor neuron degeneration and paralysis in mice [20]. More recently, ferroptosis is found to underlie the pathophysiology of heart failure resulting from doxorubicin cardiotoxicity or ischemia-reperfusion injury [21].

3.2. GPx4, Ferroptosis, and Bacterial Infection

Ferroptosis is a regulated mode of cell death executed, at least partly, via selective oxidation of membrane arachidonic acid-phosphatidylethanolamines

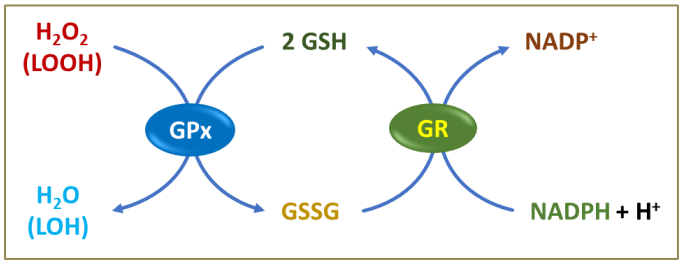

FIGURE 1. GPx-catalyzed decomposition of H_2O_2 and an organic hydroperoxide (LOOH). As illustrated, GPx isozymes, using GSH as the electron donor, catalyze the reduction of H_2O_2 and LOOH into H_2O and an alcohol (LOH), and during the reaction, GSH is oxidized to glutathione disulfide (GSSG). GSSG is reduced back to GSH via the action of glutathione reductase (GR) using NADPH as the electron donor.

(AA-PE) by 15-lipoxygenases. As discussed above, in mammalian cells and tissues, ferroptosis has been pathogenically associated with such disorders as neurodegeneration, acute renal failure, and cardiomyopathy. More recent studies also show its involvement in bacterial infection-induced host cell/tissue injury. In this regard, Darr and associates discovered that a prokaryotic bacterium—*Pseudomonas aeruginosa*, that does not contain AA-PE can express lipoxygenase, oxidize host AA-PE to 15-hydroperoxy-AA-PE, and trigger ferroptosis in human bronchial epithelial cells [22]. This finding suggested that the evolutionarily conserved mechanism of lipoxygenase-driven ferroptosis may represent a novel pathophysiological mechanism of *P. aeruginosa*-associated diseases.

Besides *P. aeruginosa*, the *Mycobacterium tuberculosis* bacteria also appear to cause cell death and tissue injury via ferroptosis, likely resulting from reduced GPx4 [23]. In an elegantly performed research study, Amaral and colleagues [23] reported that *M. tuberculosis*-induced macrophage cell death is associated with reduced levels of GSH and GPx4, along with increased free iron, mitochondrial superoxide formation, and lipid peroxidation, all of which are essential hallmarks of ferroptosis. Using mice, these investigators further showed that *M. tuberculosis*-induced acute pulmonary necrosis is associated with reduced GPx4 expression as well as increased lipid peroxidation, and the tissue injury is suppressed by ferrostatin-1, a well-characterized ferroptosis inhibitor. Notably, ferrostatin-1 treatment results in remarkable reduction of the *M. tuberculosis* bacterial load [23]. This finding is consistent with the notion that the death of infected macrophages facilitates mycobacterial spread in the lungs and other target tissues.

As GPx4 is the essential negative regulator and the most downstream component of the ferroptosis pathway [24], the findings from the above studies provide a molecular basis for targeting GPx4 to counteract bacterial infection-induced tissue injury.

4. GPX4 IN PYROPTOSIS AND POLYMICROBIAL SEPSIS

Pyroptosis is a newly discovered form of regulated cell death initiated by inflammasomes, which detect cytosolic contamination or perturbation. It occurs upon activation of proinflammatory caspases and their subsequent cleavage of a cellular protein, known as gasdermin D, resulting in gasdermin D *N*-terminal fragments that form membrane pores to induce cell lysis [25–27]. This novel mode of regulated cell death is involved in the pathophysiology of various diseases, including atherosclerosis, myocardial ischemia-reperfusion injury, microbial infections, and sepsis [26–29].

A role for GPx4 in pyroptosis and polymicrobial sepsis has been uncovered recently by Kang and associates [27]. Kang et al. reported that GPx4 and its ability to reduce lipid peroxidation negatively regulate macrophage pyroptosis and polymicrobial septic lethality in mice. They first showed that conditional GPx4 knockout in myeloid lineage cells increases lipid peroxidation-dependent caspase-11 activation and gasdermin D cleavage. The resultant *N*-terminal gasdermin D fragments then trigger macrophage py-

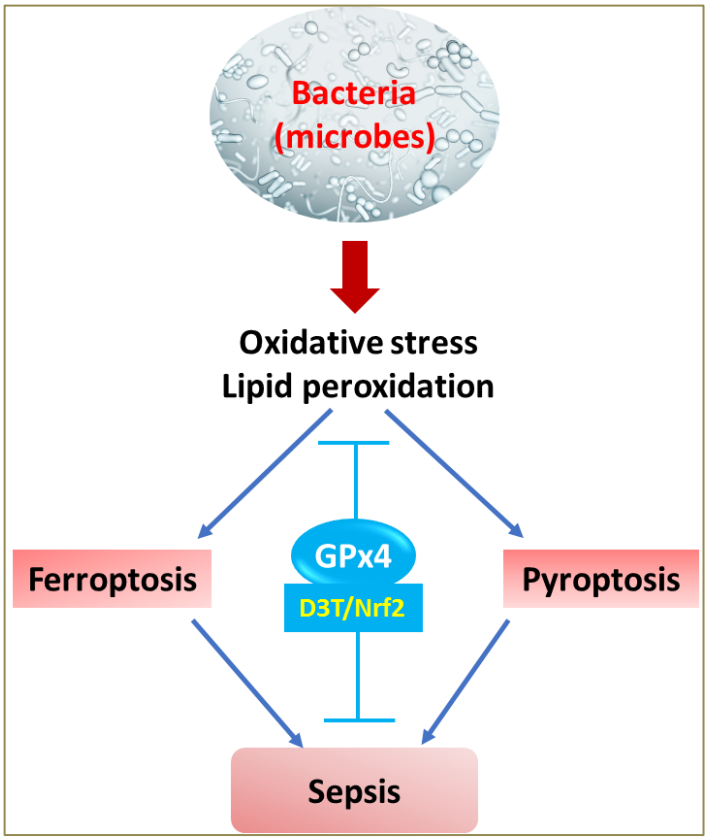

FIGURE 2. The potential role of GPx4 in protecting against sepsis. As illustrated, bacterial (other microbial) infections cause oxidative stress and lipid peroxidation, resulting in ferroptosis and pyroptosis, which in turn contribute to bacterial infection-induced tissue damage and sepsis. By suppressing lipid peroxidation and ferroptosis/pyroptosis, GPx4 may play an important role in counteracting inflammatory tissue injury and serve as a novel target for sepsis intervention. In this context, pharmacological inducers of GPx4, such as the Nrf2-activator D3T, could be developed as promising therapeutic modalities for treating sepsis as well as other pathological conditions associated with dysregulated ferroptosis and pyroptosis.

roptotic cell death in a phospholipase C gamma 1 (PLCG1)-dependent manner. They then created a polymicrobial sepsis model using GPx4-null mice and demonstrated that administration of the antioxidant vitamin E that reduces lipid peroxidation, chemical inhibition of PLCG1, or genetic caspase-11 deletion or gasdermin D gene inactivation results in suppression of lethal inflammation associated with polymicrobial sepsis in GPx4-knockout mice [27].

The study by Kang et al. for the first time revealed a protective function of GPx4 in polymicrobial sepsis via suppressing pyroptosis. The study also established a causal role for lipid peroxidation in sepsis. This lays a foundation for developing effective modalities targeting lipid peroxidation and/or boosting GPx activity to treat sepsis.

5. CONCLUSION AND PERSPECTIVES

Despite extensive research, sepsis remains the chief cause of death in intensive care units, with mortality rates ranging from 25% for the uncomplicated sepsis to 80% in those who develop multiple organ failure. Currently, there is no specific treatment of sepsis, and the only US Food and Drug Administration-

approved drug specifically indicated for treating severe sepsis, namely, drotrecogin alfa (a recombinant active protein C) was recently withdrawn following the failure of its worldwide trial, PROWESS Shock [30]. Hence, there is a great need to develop effective therapies for sepsis. In this context, advances in pathophysiology of sepsis facilitate the development of novel and effective mechanistically based therapeutic modalities for this dread disorder [31–33]. As illustrated in **Figure 2**, the identification of GPx4 as a requisite gateway to ferroptosis and pyroptosis provides a unique opportunity for developing novel strategies to control bacterial infection and combat sepsis. As GPx4 is regulated by Nrf2 signaling [34], we propose that the Nrf2 activator and GPx inducer—3*H*-1,2-dithiole-3-thione (D3T) [35, 36], and other related nutraceuticals could be developed as pharmacological GPx4 inducers for treating sepsis.

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