Advances in the Mechanism of Oxidative Stress-Induced Porcine Intestinal Injury: Postprint

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Abstract

Reproductive disorders in breeding livestock, low survival rates and high morbidity rates of young animals, and decline in livestock product quality occurring during livestock production are all associated with oxidative stress, which has become a research hotspot in animal health and nutrition. This article reviews the sources of intestinal oxygen free radicals, the mechanisms by which oxidative stress affects intestinal epithelial cell proliferation and differentiation, and the intestinal oxidative damage induced by oxidative stress in swine production.

Full Text

Preamble

Research Progress on the Mechanism of Oxidative Stress-Induced Intestinal Damage in Pigs

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Abstract: In livestock production, reproductive failure in breeding animals, low survival and high morbidity rates in young animals, and declining quality of animal products are all associated with oxidative stress, which has become a focal point in animal health and nutrition research. This review examines the sources of oxygen free radicals in the intestine, the mechanisms by which oxidative stress affects intestinal epithelial cell proliferation and differentiation, and oxidative damage to the intestine in pig production.

Keywords: oxygen free radicals; oxidative stress; intestinal epithelial cells

Redox reactions in the body are fundamental to many biochemical pathways and cellular functions [1]. Maintenance of redox homeostasis depends primarily on the dynamic balance between the oxidative and antioxidant systems. Excessive production of reactive oxygen species (ROS) or impairment of the antioxidant system disrupts this balance, leading to oxidative stress (OX) [2]. Both excessive ROS generation and insufficient ROS scavenging by the antioxidant system can cause oxidative stress, resulting in intestinal cell apoptosis and tissue damage [3]. Disruption of the intestinal mucosal barrier rapidly activates innate immunity, triggering acute inflammatory responses in the lamina propria. Once the barrier is compromised, immune cells and intestinal epithelial cells interact with pathogenic factors, producing inflammatory mediators and ROS that damage DNA, proteins, and lipids [4], ultimately activating apoptotic pathways that destroy the intestinal epithelial layer. The effects of ROS on cells are dose-dependent: low levels promote beneficial cellular responses, while high levels cause oxidative stress, cellular injury, and death. Moreover, different ROS-generating systems may elicit different responses. For example, ROS produced in mitochondria are more likely to cause cellular damage and apoptosis [5-6], whereas ROS generated at the membrane are more conducive to signaling for cell proliferation and differentiation [7]. However, this distinction is not absolute—mitochondrial ROS have also been shown to positively regulate cell proliferation, migration, and metastasis [8-9], while ROS generated by NADPH oxidase can induce apoptosis [10].

At physiological levels, ROS participate in numerous signaling pathways, including gene transcription and protein kinase activation, thereby regulating cytokine secretion and coordinating cell motility [11]. This dual nature of ROS complicates the determination of appropriate antioxidant dosages.

1 Sources of ROS Generation in the Intestine

Intestinal free radicals in animals can be classified as endogenous or exogenous based on their origin. Endogenous free radicals are primarily generated through enzymatic reactions involving the mitochondrial electron transport chain (mETC), NADPH oxidase, and xanthine oxidase. Transition metal ions in the intestine also produce free radicals via Fenton reactions. Intestinal commensal bacteria can induce ROS production in intestinal epithelial cells. Additionally, macrophages and peroxidases in the body generate free radicals. Environmental factors (high or low temperature, excessive stocking density), disease factors (bacterial or viral infections, parasites such as coccidia), and dietary factors (oxidation of unsaturated fats, mycotoxins) contribute to exogenous free radical formation and oxidative damage.

1.1 Enzymatic Reactions of mETC and NADPH Oxidase

The mitochondrial inner membrane contains mitochondrial respiratory chain enzyme complexes (MRC) composed of coenzyme Q, peripheral proteins, and cytochrome c. Electron leakage from MRC complexes I and III reduces molecular

oxygen, generating superoxide anion radicals ($O_2^{-}\cdot$) [12]. NADPH oxidase is a complex enzyme present in the plasma membrane and in macrophages (monocytes, neutrophils, and eosinophils) that produces large amounts of ROS during intestinal inflammatory responses [13]. NADPH oxidase 1 (NOX1), a member of the NADPH oxidase family, is highly expressed in colonic epithelial cells [14]. In various cell types, NOX1 plays roles in host defense, regulation of cell growth and differentiation, and cell migration [15-16]; however, its function in the intestine remains unclear and controversial. Current research indicates that NOX1 primarily protects the host against damage from interferon-γ (INF-γ) [14], lipopolysaccharide (LPS) [17], and flagellin [18], while also regulating cell proliferation and differentiation.

1.2 Other Enzymatic Reactions of Oxidases

Xanthine oxidase (XO) is found on the extracellular surface of the plasma membrane and in the cytoplasm, primarily expressed in the liver and gastrointestinal mucosa [19]. XO catalyzes the oxidation of hypoxanthine to xanthine and subsequently to uric acid during purine catabolism, with both reactions generating $O_2^{-}\cdot$. Lipoxygenase (LOX) is a non-heme iron enzyme that catalyzes the dioxygenation of polyunsaturated fatty acids, producing hydrogen peroxide (H_2O_2) derivatives. In animals, the LOX substrate is arachidonic acid (AA), and ROS are generated during its catalytic oxidation [20]. Myeloperoxidase (MPO) is a heme enzyme located in the lysosomes of neutrophils, macrophages, and monocytes that chlorinates H_2O_2 to form highly reactive hypochlorous acid (HOCl). Nitric oxide radical ($\cdot NO$) is a weak oxidant produced by nitric oxide synthase (NOS) through the oxidation of L-arginine; however, $\cdot NO$ reacts with O_2 to form peroxynitrite ($OONO^{-}$), a highly reactive oxidant that induces apoptosis in intestinal epithelial cells and reduces their proliferative renewal [21]. $\cdot NO$ and $OONO^{-}$ generate stable nitrite (NO_2^{-}) and nitrate (NO_3^{-}) that accumulate intracellularly, ultimately causing nitration and nitrosylation of macromolecules such as DNA, RNA, proteins, and lipids, thereby impairing intestinal function.

1.3 Transition Metal Fenton Reactions

Redox-active metals (such as iron, copper, chromium, and cobalt) participate in cyclic electron transfer reactions between metals and substrates, playing important roles in maintaining redox homeostasis—a phenomenon closely linked to metal homeostasis [22]. Disruption of metal homeostasis can lead to free radical-mediated DNA base modifications, enhanced lipid peroxidation, and altered thiol homeostasis [23]. Transition metal ions in the intestine can accelerate lipid peroxidation through Fenton reactions [24].

1.4 Induction by Commensal Bacteria

The intestinal microbiota provides the host with energy, stimulates immune responses, and competitively excludes pathogenic microorganisms [25]. These functions depend on "pattern recognition receptors" (PRR) that mediate host

cell-microbe interactions. PRRs consist of Toll-like receptors (TLR) and associated Nod-like receptors (NLR) that bind to motifs of "microbe-associated molecular patterns" (MAMP). PRRs represent the first line of defense against intestinal infections, activating signaling cascades that trigger innate immune responses and maintain intestinal homeostasis, serving as a hub for innate immunity [26]. Commensal bacteria (such as *Lactobacillus*) stimulate intestinal epithelial cells to produce non-pathogenic levels of ROS via NOX1 activation, thereby promoting intestinal stem cell proliferation and differentiation. Additionally, ROS activate the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, inducing upregulation of antioxidant genes [27]. Kumar et al. [28] demonstrated that *Lactobacillus* stimulates rapid ROS production in intestinal epithelial cells both in vitro and in vivo in mice treated with *Lactobacillus rhamnosus*. However, the mechanisms by which commensal bacteria induce ROS production in intestinal epithelial cells require further investigation.

2 Effects of Oxidative Stress on Intestinal Epithelial Cells

The intestinal mucosa is a complex, dynamic tissue composed of a self-renewing monolayer of surface epithelial cells and underlying components including the innate immune layer, vasculature, and other structural elements [29]. The mucosa has evolved to both digest and absorb nutrients and water while simultaneously protecting the organism from toxic substances within the intestinal lumen. This protective function depends on the continuous physiological cycle of vertical migration, differentiation, and eventual apoptosis of intestinal epithelial cells at the apical surface, followed by shedding into the intestinal lumen. This renewal and homeostasis are maintained by crypt intestinal stem cells (ISCs), which generate highly proliferative progenitor cells called transit-amplifying (TA) cells. These TA cells undergo several rounds of division and differentiate while migrating upward to the villi to perform digestive and absorptive functions, completing the intestinal cell renewal cycle every 3–7 days [30].

Among the four differentiated cell types in the villi, most are absorptive enterocytes (accounting for 80% of all epithelial cells), with the remainder being secretory cells: goblet cells, enteroendocrine cells, and tuft cells. ISCs reside primarily in the crypts, where two types have been identified: radiation-sensitive multipotent stem cells located in the crypts and crypt base columnar (CBC) cells at the crypt base [31]. Paneth cells are the only differentiated cells in the crypts, secreting numerous antimicrobial products such as lysozyme, epidermal growth factor (EGF), and transforming growth factor-β (TGF-β). They function not only in innate immunity and antimicrobial defense but also provide essential signaling for intestinal stem cells [32]. The signaling mechanisms controlling ISC self-renewal, proliferation, migration, and differentiation are not fully understood, but likely involve the Wnt/β-catenin, Notch, phosphatase and tensin homolog (PTEN)/phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt), and bone morphogenetic protein (BMP) pathways. Notably, the Wnt/β-catenin, PTEN/PI3K/Akt, and Notch pathways are highly sensitive to oxidative stress

due to their regulation by NADPH oxidase.

2.1 Wnt/β-catenin, PTEN/PI3K/Akt, and Notch Signaling Pathways

NOX1 and dual oxidase 2 (Duox2), members of the NADPH oxidase family, are highly expressed in intestinal epithelial cells [33], with NOX1 directly influencing intestinal epithelial cell proliferation and migration [34]. The canonical Wnt/β-catenin pathway is a key regulator of tissue development and homeostasis [35], and extensive in vivo and in vitro studies have demonstrated its critical role in maintaining stem cell proliferation and pluripotency [36]. Wnt signaling inactivation is triggered by N-terminal phosphorylation of β-catenin, leading to its proteasomal degradation. The phosphorylation status of β-catenin is determined by a destruction complex comprising the tumor suppressor protein APC (adenomatous polyposis coli), glycogen synthase kinase 3β (GSK3β), casein kinase I, and Axin [31]. When Wnt proteins bind to their specific Frizzled/low-density lipoprotein receptor-related protein (Frizzled/LRP) co-receptors, the destruction complex is inactivated, causing β-catenin accumulation and nuclear translocation. In the nucleus, β-catenin forms an active transcription complex with T cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors to upregulate target genes such as c-MYC and ephrin-B (EPHB) [31].

In the PTEN/PI3K/Akt pathway, PTEN negatively regulates PI3K by dephosphorylating phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to phosphatidylinositol (4,5)-bisphosphate (PIP2). Activated PI3K binds to and phosphorylates Akt in a process facilitated by phosphoinositide-dependent kinases 1 and 2 (PDK1, PDK2) [37]. The PI3K pathway can cooperate with Wnt signaling by enhancing β-catenin-mediated transcription in the nucleus, thereby strengthening intestinal stem cell self-renewal [38].

In the small intestinal epithelium, mature cells differentiate into absorptive or secretory lineages. Notch signaling promotes differentiation toward the absorptive lineage rather than the secretory lineage [39] and maintains intestinal stem cells while regulating differentiation direction to control epithelial homeostasis by targeting different progenitor cell populations [40]. Notch is a receptor protein that, along with its ligands (Jagged/Delta), is membrane-bound. Ligand binding triggers proteolytic cleavage of the receptor by the γ-secretase protease complex, activating Notch signaling [41]. This cleavage releases the free Notch intracellular domain (NICD), which translocates to the nucleus and binds to the recombination signal binding protein for immunoglobulin kappa J region (RBPJ-k) transcription factor to upregulate target genes. Differentiation of progenitor cells into enterocytes is partially determined by the transcription factor Hes1, while differentiation of secretory progenitors into goblet or enteroendocrine cells is regulated by Math1 and neurogenin 3, respectively, which are transcriptional targets of Notch signaling.

2.2 NADPH Oxidase-Mediated ROS Effects on Wnt/β-catenin, PTEN/PI3K/Akt, and Notch Signaling Pathways

NOX1 plays an important role in regulating Wnt/β-catenin and Notch signaling pathways in colonic epithelial cells. Nuclear factor- B (NF- B) is a redox-sensitive transcription factor activated by high ROS levels, and the γ-secretase protease complex that cleaves and releases NICD is regulated by NF- B. In mice with knockout of the NOX1 gene, reduced levels of the γ-secretase complex in the Notch signaling pathway decreased NICD release, leading to downregulated Hes1 expression and upregulated Math1 expression [42]. Blocking Notch signaling with γ-secretase inhibitors converts all intestinal epithelial cells into goblet cells [43]. Additionally, ROS produced by NOX1 indirectly oxidize PTEN, thereby inhibiting PI3K activation and affecting β-catenin transcription. In a rat epithelial cell oxidative stress model induced by H_2O_2, ROS induced apoptosis through the PTEN signaling pathway [44]. Furthermore, mice with NOX1 deficiency in the colon exhibited significantly reduced cell proliferation, cell cycle arrest in progenitor cells, and conversion of all progenitor cells into goblet cells through inhibition of Wnt/β-catenin and Notch signaling [42]. Numerous studies have demonstrated hierarchical regulation among the Wnt/β-catenin, PTEN/PI3K/Akt, and Notch pathways in controlling intestinal cell proliferation and differentiation [45-46].

TP53-induced glycolysis and apoptosis regulator (TIGAR) increases NADPH production and antioxidant activity by generating reduced glutathione [47]. The ratio of ATP synthesized through oxidative phosphorylation versus glycolysis is inversely proportional to TP53 expression; thus, loss of TP53 increases oxygen consumption while reducing aerobic respiration, promoting a shift to glycolysis and causing apoptosis due to elevated ROS levels. Additionally, TIGAR-deficient mice exhibit impaired intestinal cell proliferation due to increased ROS and reduced nucleotide synthesis [48]. Therefore, TIGAR provides antioxidant defense by controlling ROS levels [49]. RAC1 is a component of the NADPH oxidase signaling complex that influences multiple pathways, including mammalian target of rapamycin (mTOR), NF- B, and ROS production [50]. Similar to TIGAR deficiency, RAC1 deficiency also impairs Wnt-dependent intestinal cell proliferation, but through a different mechanism—by decreasing ROS levels [51]. This creates a paradox where both reduced and increased ROS in the intestine can lead to decreased cell proliferation.

Additionally, apurinic/apyrimidinic endonuclease 1 (APE1) is a primary factor responding to oxidative stress, widely expressed in intestinal epithelial cells and regulating various responses to bacterial infection, including chemokine production, cell proliferation, and apoptosis. APE1 can inhibit intracellular ROS production in intestinal epithelial cells by regulating RAC1-mediated NADPH oxidase. The carboxyl terminus of APE1 is responsible for repairing ROS-induced DNA damage, while the amino terminus is primarily involved in redox-mediated transcriptional co-stimulation [13].

3 Impact of Oxidative Stress on Animal Intestinal Health

In animal production, numerous factors cause ROS accumulation and oxidative stress, including thermal stress, weaning stress, stocking density, mycotoxins, and oxidized fats in feed. Oxidative stress is associated with many syndromes, with intestinal oxidative damage being particularly susceptible and harmful. The digestive tract functions in nutrient digestion and absorption while also serving immune, endocrine, and mucosal barrier roles. The structural integrity, redox status, microbial flora, and enzymatic balance of the gastrointestinal tract are critical for its normal physiological function. Apoptosis of intestinal epithelial cells is essential for epithelial turnover and tissue stability, and abnormal apoptosis can lead to mucosal barrier damage and gastrointestinal dysfunction [52]. In pig production, weaning is a critical process for piglets due to substantial dietary and environmental changes. The prominent morphological changes in the intestine caused by weaning stress include villus shedding, villus shortening, and crypt hyperplasia [53]. Zhu et al. [54] weaned 96 piglets from 12 litters and found that weaning stress significantly reduced villus height, increased crypt depth, compromised intestinal barrier function, promoted free radical generation, inhibited antioxidant effects, and decreased digestive enzyme activity.

Apoptosis occurs through two main pathways: the intrinsic (mitochondria-dependent) and extrinsic (Fas-dependent) pathways. The intrinsic pathway is mediated by mitochondria and characterized by activation of cysteine-aspartic protease-9 (Caspase-9) [55], while the extrinsic pathway involves cysteine-aspartic protease-8 (Caspase-8), which is activated through membrane apoptosis receptors such as Fas [56]. Both pathways converge on the common execution phase of apoptosis, requiring proteolytic activation of cysteine-aspartic protease-3 (Caspase-3) to trigger caspase activation [57]. Zhu et al. [58] reported that weaning stress enhanced free radical generation, significantly increasing expression of Fas, Caspase-3, and Caspase-9 genes, indicating that weaning-induced stress increases apoptosis in weaned piglet intestinal epithelial cells by activating both intrinsic and extrinsic apoptotic pathways. Therefore, oxidative stress not only causes intestinal mucosal cell damage and impairs digestive and absorptive functions but also influences cell proliferation, differentiation, and apoptosis through ROS-mediated signal transduction in intestinal epithelial cells.

4 Conclusion

In pig production, when pigs experience stress or disease, abnormal metabolism leads to sudden ROS overproduction. The intracellular level of ROS determines its ability to induce cell proliferation and differentiation, cytokine release, apoptosis, and innate immune responses. The intestine constantly interacts with the external environment and has the most frequent contact with external microorganisms. Consequently, excessive ROS accumulation disrupts the redox balance and antioxidant enzyme systems, causing intestinal mucosal redox imbalance and disease development. The dual nature of ROS makes antioxidant

dosage determination challenging. Future research should focus on understanding the causes and mechanisms of oxidative stress, investigating how different types and levels of free radicals affect intestinal damage in animals, and rationally applying antioxidants in animal production according to specific oxidative stress conditions.

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