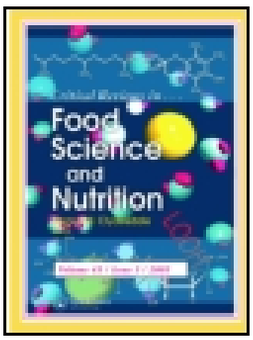

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REVIEW

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Mechanism and intervention measures of iron side effects on the intestine

Xiao Qi[a,b], Yuanxiao Zhang[b], Hang Guo[b], Yu Hai[a], Yane Luo[a], and Tianli Yue[a,c]

[a]College of Food Science and Technology, Northwest University, Xi'an, Shaanxi, China; [b]School of Chemical Engineering, Northwest University, Xi'an, Shaanxi, China; [c]Ministry of Agriculture, Laboratory of Quality and Safety Risk Assessment for Agro-products (Yangling), Beijing, China

ABSTRACT

Excess oral iron in the intestinal tract usually produces reactive oxygen species via Fenton and Haber-Weiss reaction, so oxidative stress is triggered. Lipid peroxidation procedurally appears, ferroptosis, apoptosis and necrosis are often induced, subsequently, mitochondrial damage, endoplasmic reticulum dysfunction and even cell death occur. As a result, the intestinal epithelial cells are destroyed, leading to the incompleteness of intestinal mechanical barrier. Simultaneously, iron supplement can change the compositions and metabolic processes of intestinal microbes, and the intestinal inflammatory may be worsened. In principle, the easier dissociation of Fe^{2+} from oral iron supplements is, the more serious intestinal inflammation will occur. Fortunately, some interventions have been developed to alleviate these side effects. For instance, some antioxidants e.g. VE and ferulic acid have been used to prevent the formation of free radicals or to neutralize the formed free radicals. Furthermore, some new iron supplements with the ability of slow-releasing Fe^{2+}, e.g. ferrous citrate liposome and EDTA iron sodium, have been successfully prepared. In order to recover the intestinal micro-ecological balance, probiotics and prebiotics, bacterial consortium transplantation, and fecal microbiota transplantation have been developed. This study is meaningful for us to develop safer oral iron supplements and to maintain intestinal micro-ecological health.

KEYWORDS

Oral iron; Side effects; Oxidative stress; Intestinal barrier; Intestinal inflammation

1. Introduction

It is well known that iron is one of the essential trace elements in the human body and it is closely related to erythropoiesis. Iron plays an important role in the metabolism of various organs and even the whole life cycle. There is generally 4 to 5 g of iron in adult body, present as functional, stored or transported iron. About 2.5 g of iron exists in the form of hemoglobin to participate in oxygen transport. And the rest exists in the form of ferritin. Ferritin usually exists in the spleen, liver, and bone marrow, acting as a buffer against iron deficiency and iron overload (Reizenstein 1991; Mesías et al. 2013; Allen 2002; Imam et al. 2017; Walter et al. 2002). The lack of iron can induce nutritional deficiency, and serious iron deficiency will cause iron deficiency anemia (Silva Neto et al. 2018). About 2 billion people suffer from iron deficiency anemia in the world (Galloway and Mcguire 1994), covering almost all ages, such as all infants, preschoolers, pregnant women, adolescents, premenopausal women, and the elderly (Palacios 2011). Now it has been approved that iron supplements are effective to treat iron deficiency. So, iron supplements are one of the necessities of daily life in most families (Allen 2002; Lopez et al. 2016; Tripathi and Platel 2011; Mesías et al. 2013).

Iron supplements include oral iron and intravenous iron. Oral iron supplements are commonly ferrous sulfate, ferrous fumarate, iron gluconate, ethylene diamine tetraacetic acid (EDTA) iron sodium, iron dextran, etc.; intravenous iron agents include maltose iron, iron dextran, iron oxide nanoparticles, carboxyl maltose iron, isomalt iron, glucose iron, etc. Generally, intravenous iron is applied to the case of severe iron deficiency, most people would like to use oral iron supplements with good compliance and moderate price (Galloway and Mcguire 1994; Lopez et al. 2016). It has been proved that oral iron can effectively supplement iron, but many of them have been found to produce adverse effects if they are incorrectly used (Hallberg et al. 1966; Reeves and Yip 1985; Schümann et al. 2007). For example, if oral iron is taken more than 120 mg/d (Werner et al. 2011), teeth and feces will be blackened, and even Alzheimer's disease (AD) and inflammatory bowel disease (IBD) will be induced. The common side effects of oral iron supplements on animals and human beings are respectively listed in Tables 1 and 2. Among these side effects, diarrhea is the commonest symptom (Millar et al. 2000; Heimbach et al. 2000). As to the children younger than 5 years old, diarrhea is the second killer, and the number of child death from diarrhea in 2013 was as high as 577 thousand (Lin et al. 2018). Beside diarrhea, when the dose of ferrous sulfate exceeds 60 mg/kg, ferrous sulfate often engenders serious toxicity or death (Chen et al. 2016). In addition, chronic diarrhea can emerge malnutrition, which affects the growth and development of adolescents and degrades people's quality of life.

CONTACT Yane Luo luoyane@nwu.edu.cn College of Food Science and Technology, Northwest University, Xi'an, Shaanxi 710069, China.
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Table 1. Side effects of oral iron supplements on animals.

Name of oral iron supplements	Animal model	Dosage	Side effects	The internal causes of side effects	References
$FeSO_4$	C57BL/6 mice	500 ug/g body weight	Diarrhea, decreased activity, erect hair, trembling and curling of the body	Shed, shortened, and loosely arranged of intestinal villi, and intestinal mucosa erosion in ileum; swelling of endoplasmic reticulum and disappearance of ribosome in intestinal cells	Lin et al. (2018)
	SD rat	8000 μg/d	Increase of exhaled ethane and MDA content	Increase of lipid peroxidation in kidney and liver	Knutson et al. (2000)
			RCR reduction	Hepar mitochondrial DNA damage	Walter et al. (2002)
Pentacarbonyl iron	Wistar rat	3 g Fe/kg diet	Loss of appetite, weight loss, worsened colitis	Deformation of intestinal crypt, obvious damage of intestinal epithelial cells, erosion of intestinal mucosal, and the increase of lipid peroxidation level	Carrier et al. (2002)
Ferrous fumarate	Wistar rat	1.82 mg/(kg body weight·d)	Weight loss and worsened colitis	Disappearance of entire crypt and the increase of lipid peroxidation levels in colon	Erichsen et al. (2005)
Ferric ammonium citrate	Wistar rat	5 mg Fe/100 g body weight	Decrease of SOD level while increase of MDA level	Increase of lipid peroxidation level in serum	Xu et al. (2014)

Abbreviations: MDA, malondialdehyde; RCR, respiratory control ratio; SOD, Superoxide Dismutase.

Table 2. Side effects of oral iron supplements on human.

Name of oral iron	Dosage (mg Fe/d)	Side effects	Study
$FeSO_4$	60	Diarrhea	Souza et al. (2009)
	98	Lipid peroxidation, increase of plasma MDA content and the rate of exhaled ethane	King et al. (2008)
	200	Nausea, diarrhea and constipation	Tolkien et al. (2015)
Ferrous fumarate	120	Abdominal pain and diarrhea	Erichsen et al. (2005)
	200	Nausea, vomiting and gastritis	Bhavi and Jaju (2017)
	222	Nausea, vomiting, heartburn, diarrhea, fecal black, constipation, epigastric pain	Hallberg et al. (1966)
Ferrous gluconate	87	Nausea, black stool, constipation and diarrhea	Munim and Rashid (2017)
	222	Nausea, heartburn, constipation, diarrhea and epigastric pain	Hallberg et al. (1966)
Ferrous glycine sulfate	100	Nausea, vomiting and constipation	Abbas et al. (2018)
	222	Nausea, heartburn, constipation, diarrhea and epigastric pain	Hallberg et al. (1966)
Ferrous succinate	65	Nausea and vomiting	Cao et al. (2011)

Commonly, oral iron supplements are digested and absorbed by the gastrointestinal tract, and the main sites of absorption are the small intestine. Oral iron is transported through intestinal barrier and carried to various tissues and organs along with the blood circulation, and then it functions as a nutrient or induces side effects at cell level. Improper iron compositions or using doses usually trigger oxidative stress (OS) (Knutson et al. 2000), and then cascading destroys and even death of cells happen. With regard to intestine, cell death is easy to break the intestinal integrity. As a result, the physical barrier of the intestinal tract (i.e., intestinal mucosal epithelial structure) is wrecked by oxidative reactions. With the interactions between cells and surrounding conditions, the chemical barrier, immune barrier and biological barrier of intestine will be undermined, and intestinal dysfunction appears (Baumgart and Dignass 2002; Sina et al. 2018; Morgan et al. 2012).

With the widespread use of oral iron supplements, their side effects have become non-negligible and inescapable. In order to alleviate and even evade the side effects arising from oral iron, it is required to clarify the interactions between intestinal cells and oral iron supplements. Although the side effects of oral iron have been frequently reported, the intrinsic effect mechanism of oral iron is scarcely summarized (Lin et al. 2018). This article is focused on the side effects of iron supplements on the gastrointestinal tract, the intrinsic mechanisms were analyzed, such as the synthesis pathways of ROS, the effect mechanisms of oral iron on intestinal cells and flora. Particularly, the molecular mechanisms of cell damage and death owing to excess iron were illustrated. Based on the effect mechanisms of oral iron, some possible intervention measures to alleviate oxidative stress and to recover the micro-ecological balance in intestinal tract were proposed, which would pave a foundation for further optimization of iron composition and application.

2. Mechanism of side effects of oral iron supplements on the intestine

2.1. The main pathways to generate hydroxyl radicals

The first important step for oral iron supplements to play their roles is to cross the intestinal barrier. Oral iron arrives at the intestine and firstly contact with physical barrier and chemical barrier, then a series of biochemical reactions occur. Especially, the free metal divalent ions in cell, Fe^{2+} can react with hydrogen peroxide, generating hydroxyl

radicals (OH·) via the Haber-Weiss reaction (Klaassen and Amdur 2013; Kehrer 2000; Harris et al. 1992). The reaction equation is described as following:

$$O_2- + H_2O_2 = O_2 + OH^- + OH$$

Iron can acts as a catalyst to react with superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) in phagocytes (e.g. neutraphils, eosinophils and monocytes) to generate efficient of superoxide radical, meanwhile, hydroxyl radicals (OH·) can also be produced (Babior and Peters 1981; Carrier et al. 2002). These reactions are termed as Fenton reactions (Carrier et al. 2002), and the details are listed as following:

$$Fe^{3+} + O_2 = Fe^{2+} + O_2^-$$
$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^- + OH$$

It is well known that hydroxyl radical is very active, it can attack the polyunsaturated fatty acids (PUFAs) of phospholipid bilayer with increasing the permeability of the membrane (Carrier et al. 2002) (Figure 1), oxidize the residues of amino acids, attack and change the deoxyribonucleic acid (DNA) bases in mitochondria and nucleus with breaking DNA double strands (Reizenstein 1991).

2.2. Effect of excess iron on intestinal cells

2.2.1. Lipid peroxidation of cells caused by iron

Several studies have testified that excess oral irons can oxidize lipids. For instance, Knutson et al. found that daily feeding 8000 μg of ferrous sulfate to normal Sprague-Dawley rats still increased malondialdehyde (MDA) level by 1.6-fold (Knutson et al. 1992). And they also had authenticated that a daily iron supplement of 98 mg of iron in ferrous sulfate to women, plasma MDA and breath ethane exhalation rates (BEER) were increased by more than 40% after 6 weeks of supplementation (King et al. 2008). Benedet et al. demonstrated that iron, chromium, lead and cadmium participated in Fenton reaction, resulting in an increase of lipid peroxidation level (Benedet and Shibamoto 2008).

Indeed, polyunsaturated fatty acids (PUFAs) are easy to be oxidized, particularly, long-chain fatty acids containing a variety of double bonds, such as arachidonic acid (AA), linoleic acid (LNA) and docosahexaenoic acid (DHA). The (1Z, 4Z) pentadiene structure of the PUFAs is the easiest part to be oxidized. PUFAs peroxides abstract hydrogen atoms from the methylene group bridging two double bonds at first, since the bond energy of C-H bond in bisallyl radical is so week that hydrogen atom is easily removed (Figure 1B). Meanwhile, the resonance π-system can assist lipid to convert into a more stable isomerization form, so lipid is easy to react with hydroxyl radicals to form lipid peroxides and the conjugated (1Z, 3E) diene structure (Gaschler and Stockwell 2017).

The breakdown process of lipids via Fenton reaction is shown in Figure 1C. The degraded products of AA, 4-Hydroxynonenal (4-HNE) and MDA all contain an active aldehyde, aldehyde can be cross-linked with amino groups of membrane proteins or DNA to form dialdehyde (Esterbauer et al. 1991; Gürbüz and Heinonen 2015). As a result, the structures and functions of membrane proteins are changed, the permeability of cellular membrane is enhanced (Esterbauer et al. 1991). In addition, lipid peroxidation of intestinal mucosal epithelial cells induced by oxygen free radicals not only cripples the stability of cellular and organelle membranes, but also initiates a death program, ferroptosis (Feng et al. 2018) (Figure 1A). Some researchers have proved that non-chelating irons, such as ferrous sulfate and iron citrate, are easier to trigger ferroptosis of intestinal cells (Angeli et al. 2017).

Normally the intestinal cells usually form an integral and tight physical barrier to protect the intestinal tract, the death of intestinal cells can undermine the intestinal physical barrier and even the other barriers (Xie et al. 2016). It is well accepted that the intestinal barrier can be divided into four layers, i.e., physical barrier, chemical barrier, immune barrier and biological barrier (Soderholm and Perdue 2001). The intestinal barrier is known as the protector of our bodies, and its integrity is the key factor to determine intestinal functions (Swank and Deitch 1996; Kong et al. 2008). If the basic physical barrier constructed by the tight junction of intestinal epithelial cells is destroyed, it will change chemical barrier and ruin immune barrier, the intestinal dysfunction emerges, such as enteritis (Baumgart and Dignass 2002; Sina et al. 2018; Maloy and Powrie 2011; Morgan et al. 2012). Carrier *et al* also proved found that pentacarbonyl iron (270 mg/kg) could worsen inflammatory of mice and contribute to severe intestinal bleeding (Carrier et al. 2002).

2.2.2. Mitochondria damage aroused by excess iron supplements

Mitochondrion is an organelle for aerobic respiration, and is known as the "power station" to realize energy conversion (Enrique and Kelvin 2000), oxidative phosphorylation as well as calcium-ion storage. It has a bilayer membrane structure and is also related to the survival of cells (Martinou and Green 2001). As reported, it is found that Sprague-Dawley rats fed with 8000 μg of $FeSO_4$ no matter of iron-deficient anemia or not appeared serious mitochondrial damage and low respiratory rate (Walter et al. 2002). Excess irons usually participate in Fenton reaction and Haber-Weiss reaction. Immoderate hydroxyl radicals are generated, triggering some death pathways inside and outside mitochondria (Figure 2), e.g. apoptosis and necrosis.

Oxidative stress can be induced when ROS formed by the Fenton reaction is not removed in time, mitochondrial permeability transition pore (MPTP) is opened via forming Bax (Bcl-2-associated X) and Bak (Bcl-2 homologous antagonist killer) proteins on mitochondrion (Mikhailov et al. 2001; Von Ahsen et al. 2000), or voltage-dependent anion-selective channel (VDAC) is opened by these free radicals. Subsequently, water and protons in cytoplasm enter into mitochondria, causing the mitochondria to swell and even rupture. Further, cytochrome C (Cytc) and apoptosis inducing factor (AIF6) are released from mitochondria (Huang et al. 2000; Kroemer et al. 2007). Cytochrome C forms a complex with apoptotic protein caspase 9 and Apaf-1 (Zhivotovsky et al. 1998; Desagher and Martinou 2000), and

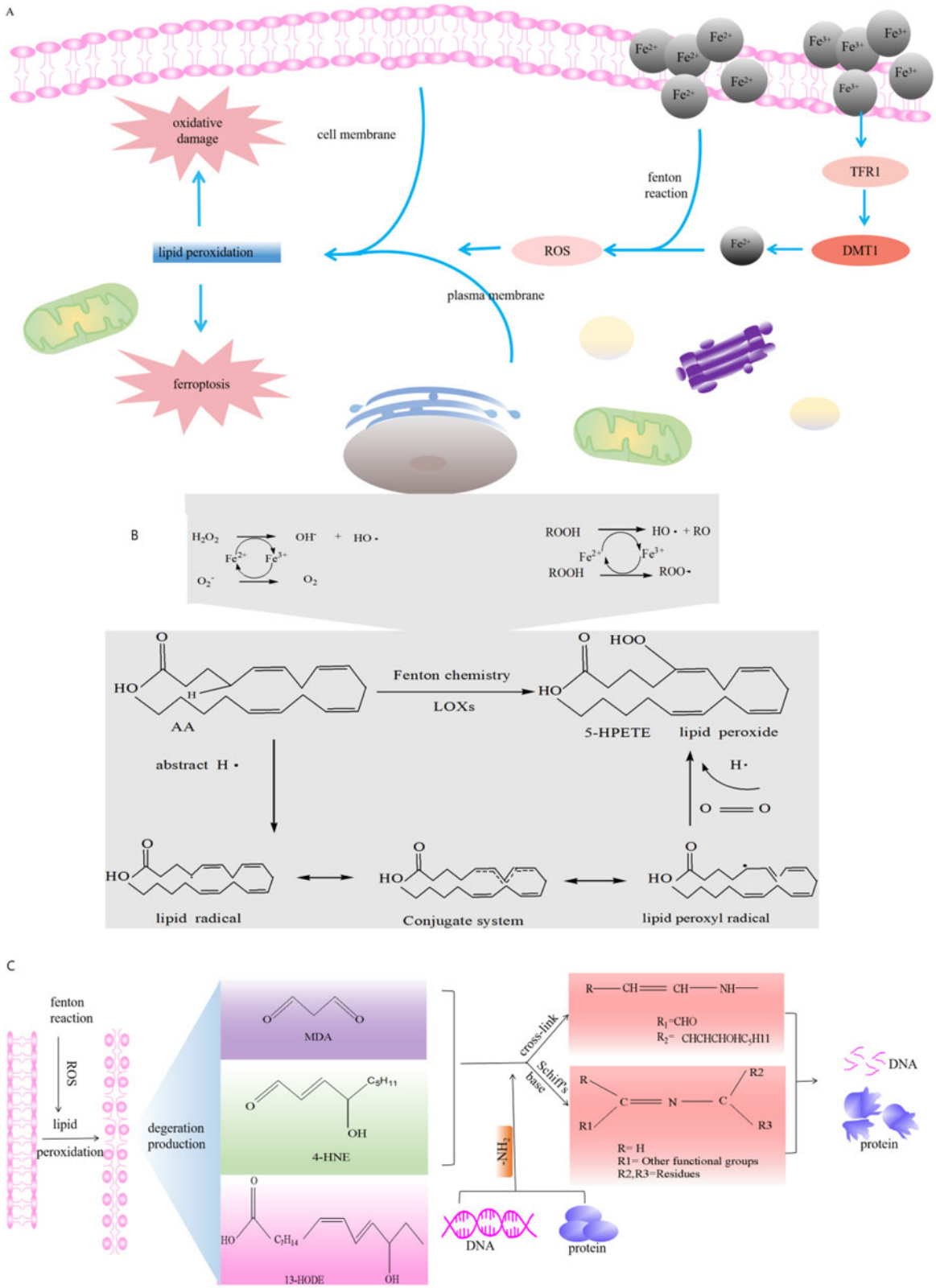

Figure 1. Picture A shows that free irons in the intestine produces oxidative damage to the cellular and organelle membrane via Fenton reaction with forming active oxides. Picture B presents the process of lipid peroxidation with arachidonic acid (AA) acted as a model. Picture C shows the effects of lipid peroxides on DNA and proteins.
Abbreviations: TFR1, transferrin receptor1; DMT1, divalent metal transporter 1 13-HODE, 13-hydroperoxyoctadecadienoic acid.

they activates caspase 3 and caspase 7 to promote apoptosis (Li et al. 1997). Apoptosis inducing factor (AIF) enters into the nucleus and causes nuclear DNA damage, contributing to cell death and intestinal dysfunction (Brenner and Mak 2009). Since these intrinsic pathways of apoptosis mentioned above have been initiated, mitochondrial respiratory and electronic chains are repressed, and then energy supply is weakened (Wang and Youle 2009).

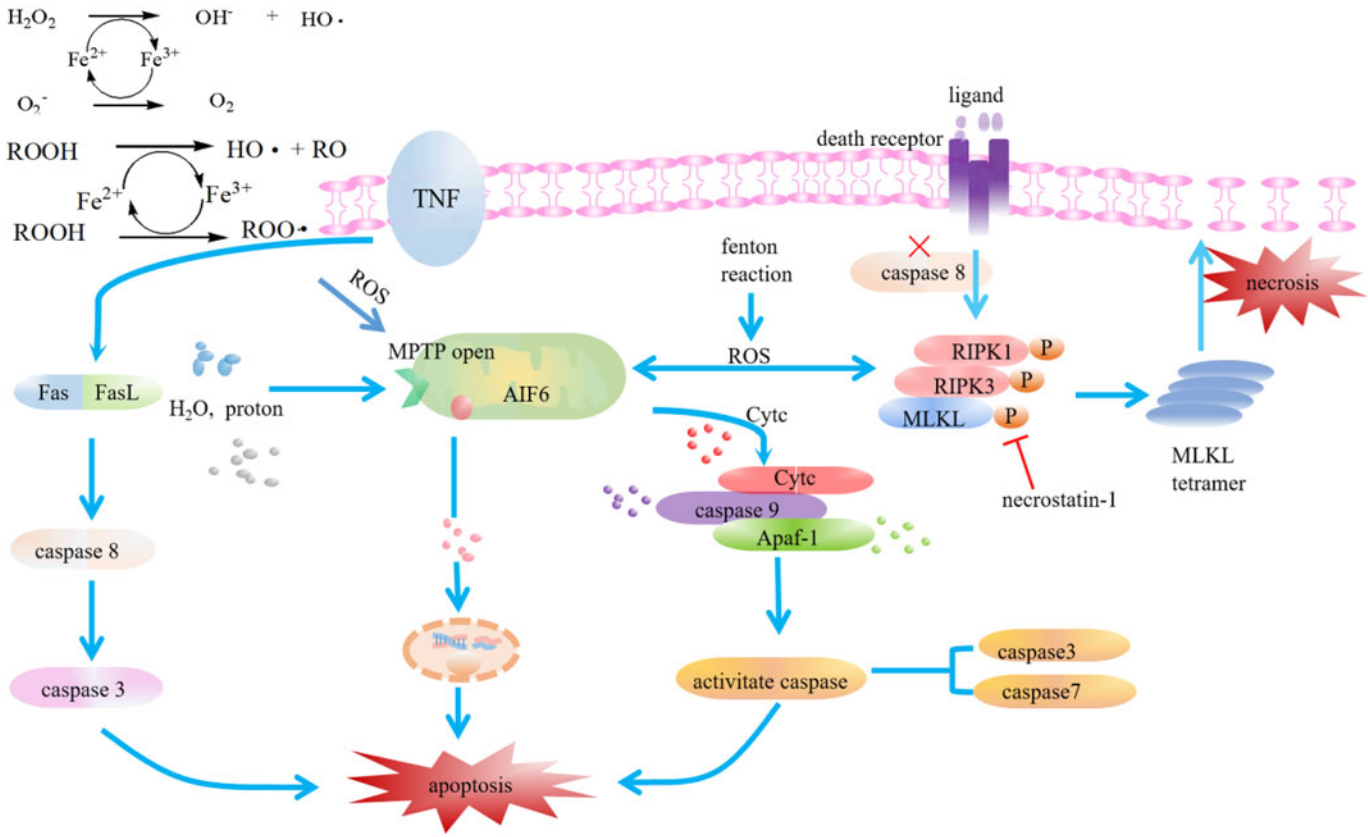

Figure 2. The mechanisms of mitochondrial apoptosis initiated by ROS.
Abbreviations: TNF, tumor necrosis factor; MPTP, mitochondrial permeability transition pore; AIF6, apoptosis inducing factor; Cytc, cytochrome C; Apaf-1, apoptotic protease activating factor-1; Fas, Apo-1/CD95; FasL, Fas-Ligand; RIPK1, receptor interacting protein kinase 1; RIPK3, receptor interacting protein kinase 3; MLKL, mixed lineage kinase domain-like.

Beside the intrinsic apoptotic pathways of mitochondria, the extrinsic apoptotic pathway involving tumor necrosis factor (TNF) also exists (Figure 2). TNF receptor family, including Tumor necrosis factor receptor 1 (TNFR1), FAS (Apo-1/CD95), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL-R1), does activate caspase-8 existing in cytoplasm or on membrane of mitochondrion, simultaneously initiates caspase cascade, and executes death signal transport program (Brenner and Mak 2009; Peter and Krammer 2003; Stegh et al. 2002). If Caspase-8 is inactivated, ROS activates death receptors e.g. T-cell receptor and Toll-like receptors, then receptor interacting protein kinase 3 (RIPK3) is phosphorylated, accompanying the phosphorylation of RIPK1 and mixed lineage kinase domain-like (MLKL). The MLKL tetramer which can enhance the permeability of plasma membrane is formed, and then cell necrosis will be triggered (Fearnhead et al. 2017).

In addition, the abundant lipid peroxides and hydroxyl radicals produced by excessive iron supplementation can oxidize unstable guanine bases to mutate DNA (Helbock et al. 1999), with destroying mitochondria (Kehrer 2000; Klaunig et al. 1998). Mitochondrial dysfunction tightly impacts intestinal cell survival. Being similar to the negative effects of excess iron on lipid peroxidation, the adverse side effects from iron-induced oxidative stress on mitochondria do harm to intestinal barriers, and induce or worsen intestinal inflammation (Szentkuti et al. 1990; Benedet and Shibamoto 2008).

Surprisingly, iron deficiency may decrease hemoglobin concentration but increase mitochondrial fragility, so that hydroxyl radicals (OH·) in body cannot be scavenged timely, lipid peroxidation levels are further increased in iron-deficient mice. Besides, mitochondrial uncoupling (Cadenas and Davies 2000) can increase the content of reactive oxygen species (ROS), which further aggravate mitochondrial DNA damage in liver cells, neutrophils and lymphocytes (Auerbach et al. 2013). Furthermore, iron deficiency can increase copper absorption, decrease ribonucleotide reductase activity, change cellular homeostasis, etc., which will motivate mitochondrial dysfunction and further aggravate lipid peroxidation.

2.2.3. Endoplasmic reticulum stress stimulated by ROS

The endoplasmic reticulum (ER) is the second largest synthetic place of sugars, proteins, and lipids. Since it is also involved in protein modification, processing, and assembly, folding and transporting of nascent peptides, it plays a significant role in life processes.

Now, it has been proved that endoplasmic reticulum stress (ERS) can aggravate intestinal inflammation (Seervi 2018). Werner *et al* found that when the rats were intraperitoneally injected with 90 mmol/g of FeTNA (i.e., $Fe(NO_3)_3$

complexed with nitrilotriacetic acid at a ratio of 1:2) per week, their intestinal tissues were not damaged, and the number of probiotics in the intestines increased; however, if 720 mg/d of ferrous sulfate was used, it brought about intestinal villus atrophy and intestinal gland damage in rats. On the bottom wall of intestine, a large number of lymphocytes reside. The impaired mitochondria and endoplasmic reticulum, no matter of lymphocytes or intestinal epithelial cells, can wreck immune homeostasis and trigger ERS, which touch off intestinal dysfunction and give rise to the occurrence or aggravation of inflammatory diarrhea (Knutson et al. 2000; Tolkien et al. 2015; Rush et al. 1985). If irons activate the nuclear factor kB (NF-kB), intestinal inflammation will be further deteriorated (Knutson et al. 2000). Lu et al. discovered that ERS was intensified *in vivo* by excessive iron, and the amount of proteins related to apoptosis was up-regulated (Lu et al. 2011; Tan et al. 2013; Ma et al. 2004). As shown in Figure 3, ERS stimulates the formation of unfolded proteins, makes endoplasmic reticulum overload and activates caspase-12-mediated apoptotic pathways, while the expression of endoplasmic reticulum chaperones such as glucose-regulated protein (including GRP78, GRP94) are stimulated in order to reduce ERS (Malhotra and Kaufman 2007; Tabas and Ron 2011; Hamman et al. 1998). Unfortunately, the stress response cannot be regulated in an unbalanced state. In this case, protein kinase R-like ER kinase (PERK) (Ohoka et al. 2005), the serine/threonine-proteinkinase/endoribonuclease inositol-requiring enzyme 1 (IRE1) (Kouroku et al. 2007; Urano 2000) and AIF6 (apoptosis inducing factor 6) are activated (Haze et al. 1999), cell apoptosis automatically initiates (Werner et al. 2011; Oyadomari et al. 2002). Finally, ERS promotes intestinal cell damage and worsens intestinal inflammation.

2.3. Effects of iron on intestinal flora

The human intestine is the habitat of microorganisms, and there are about 10^{14} kinds of bacteria, and the number of microorganisms is as 13 times as that of human cells. The intestinal flora constitutes the intestinal biological barrier (Huang et al. 2017; Leser and Mølbak 2009; Paul et al. 2015), and they help nutrient molecules to be absorbed, regulate metabolism, correct the immune system, and protect the host from pathogens (Eckburg et al. 2005).

2.3.1. Composition change in intestinal flora

There are many probiotics and pathogens to form the intestinal flora in the intestine, and their number ratio is very important to human health (Gareau et al. 2010). When the probiotic species are dominant, their growth and metabolism will promote gastrointestinal motility, benefit the synthesis of vitamins, rapidly discharge harmful substances and prevent the invasion of pathogens; while pathogenic bacteria increase so much to break healthy and stable balance, constipation, diarrhea, or reabsorption and utilization of harmful substances may occur, then pathogens are easily invaded. When the number of probiotics is about as 1,000 to 10,000 times as that of pathogenic bacteria, the balance of the intestinal flora can be reached, and the intestinal tract is healthy. Therefore, the balance and compositions of the intestinal

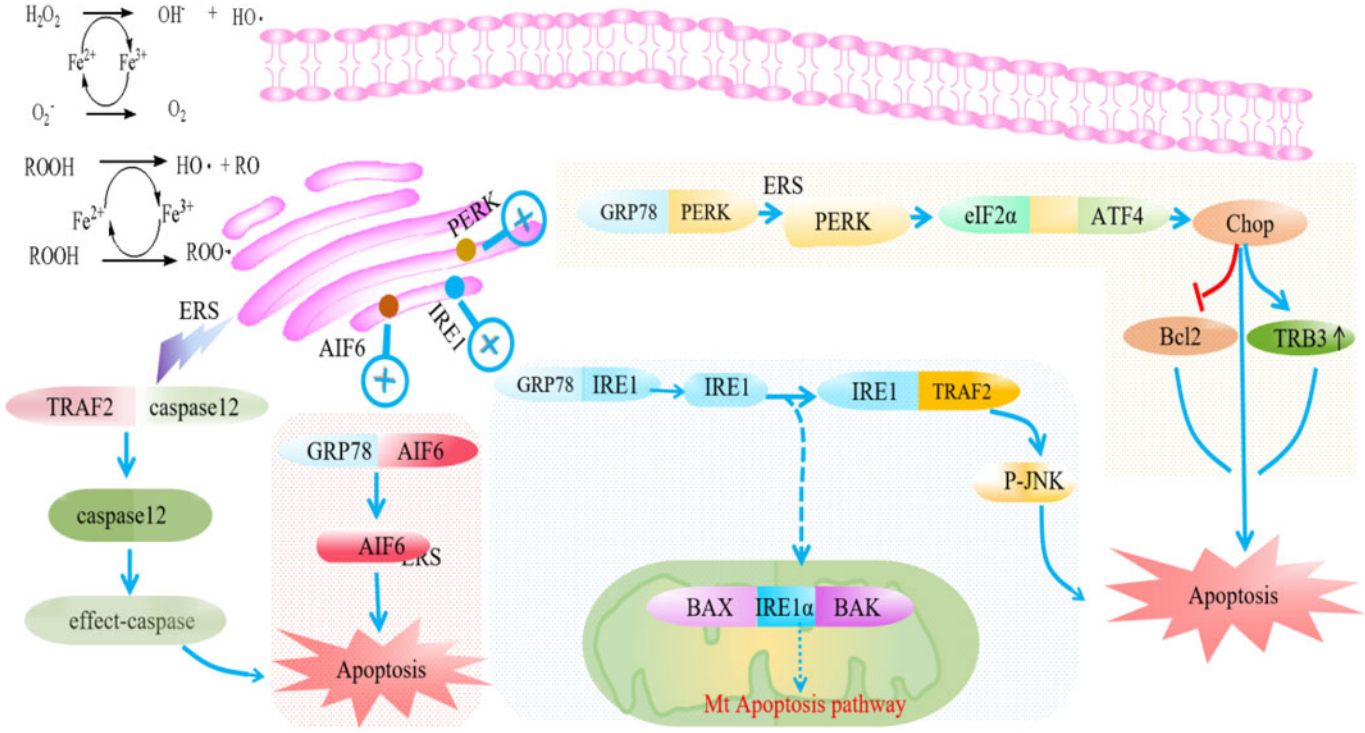

Figure 3. Mechanisms of apoptosis related to ERS induced by ROS. Four apoptotic pathways involving endoplasmic reticulum are respectively mediated by PERK, AIF6, IRE1, and TRAF2.
Abbreviations: TNF, tumor necrosis factor; MPTP, mitochondrial permeability transition pore; AIF6, apoptosis inducing factor; Cytc, cytochrome C; Apaf-1, apoptotic protease activating factor-1; Fas, Apo-1/CD95; FasL, Fas-Ligand.

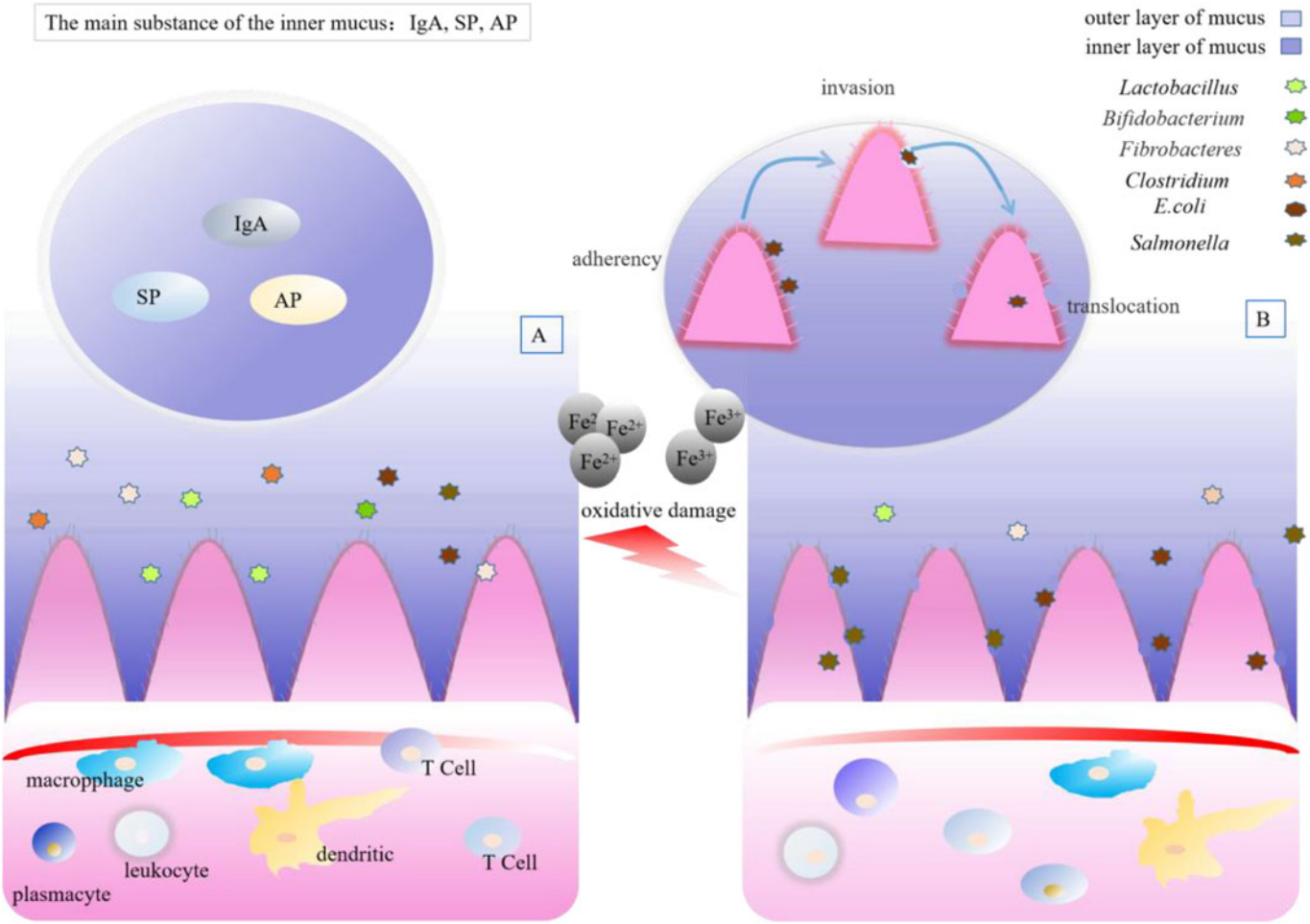

Figure 4. A schematic representation of peroxidative damage to intestinal cells and changes in intestinal flora due to excessive oral iron supplement. Picture A shows a schematic diagram of the intestinal micro-ecology of a healthy host. Picture B depicts the shrink and ablation of intestinal villi resulting from taking an excess of iron.
Abbreviations: IgA, immunoglobulin A; SP, scavenger molecules; AP, antimocrobial peptide.

microecology is particularly important for human health (Farhadi et al. 2003).

Some reports have shown that excessive oral iron supplements can damage the intestinal micro-ecology. High doses of ferrous sulfate changed the compositions of intestinal microbes (Lin et al. 2018), the number of probiotics e.g. *Lactobacilli* and *Bifidobacteria* was decreased, and the number of some pathogenic enterobacteria, such as *Escherichia coli* and *Salmonella typhimurium*, was increased. Pathogenic enterobacteria *E. coli* can specially attach and alter the cytoskeleton and apical surface of host cells via the type III secretion system, disrupt the tight junction proteins on intestinal wall, and cause intestinal damage (Baumgart and Dignass 2002). *Salmonella typhimurium* is well known to induce food poisoning and inflammatory bowel disease, the increase of *Salmonella* will unavoidably worsen intestinal inflammation (Winter et al. 2010). Lin et al. found that high doses of ferrous sulfate (500 ppm) could seriously damage intestinal tissues, crack intestines and loose intestinal villi, thereby, half of the mice died (Lin et al. 2018). It has been testified that unabsorbed oral iron supplements, except for systemic iron *in vivo*, provides the essential nutrients for pathogenic microorganisms to enhance their content and virulence (e.g. adhesion, invasion and translocation), and the proportion of dominant intestinal strains is reduced, intestinal diseases appeared (Lin et al. 2018; Werner et al. 2011).

The oral iron supplement that is easier to dissociate Fe^{2+} from water-soluble is more beneficial for the absorption of intestinal pathogenic bacteria, correspondingly, the side effects, such as nausea, vomiting and diarrhea, are also more serious. For example 10 mol/L ferrous citrate promoted the growth of pathogenic bacteria, such as *Salmonella typhimurium*, *Escherichia coli* and *Citrobacter freundii*; moreover, pathogenic *S. typhimurium* and *E. coli* and conditional pathogenic *C. freundii* have strong adhesion, invasion, translocation and colonization ability (Kortman et al. 2012). Contrarily, the adhesion ability of probiotics e.g. *Enterococcus faecalis* and *lactobacilli* slightly decreased with a slight increase of translocation ability (Kortman et al. 2012). As show in Figure 4, *Escherichia coli* and *Salmonella* in the case of high iron content have strong translocation capacity and colonization ability on the mucosa, which are easy to break the balance between probiotics and pathogens (Werner et al. 2011). In fact, the destroy of the beneficial bacteria and the enhanced toxicity of the harmful bacteria all do harm to intestinal epithelial cells, and then intestinal inflammation and other intestinal diseases occur owing to excessive iron (Harris et al. 1992; Kortman et al. 2012).

If the dosage of iron supplements is not enough to impact the dominant probiotics, the intestinal epithelial cells will be intact and healthy. When the excessive amount of iron is taken, the beneficial bacteria will be decreased, while the virulence of the pathogenic microorganism will be increased; consequently, the integrity of the intestinal cells is destroyed, the intestinal barrier function is disordered, and the intestinal inflammation is deteriorated (Figure 4).

2.3.2. Changes of metabolic pathways and products of intestinal flora

The metabolic products of intestinal flora are closely related to micro-ecologic health. For instance, *Bifidobacteria* and *Lactobacillus* can synthesize a variety of B vitamins (B1, B2, B6, B12, etc.), vitamin K, niacin and pantothenic acid (LeBlanc et al. 2013, 2017; Deguchi et al. 1985), these metabolites are necessary for human growth and development; *Bifidobacteria* and *Lactobacillus* can also form essential amino acids through degraded proteins, and promote the absorption of mineral elements, such as iron, magnesium and zinc. Dostal et al. demonstrated that iron fortification altered the metabolic pathways and products of intestinal flora, 10 ppm and 20 ppm iron significantly reduced the concentration of propionate and butyrate in the feces (Dostal et al. 2012). Butyric acid is a short-chain fatty acids (SCFAs) formed by the fermentation of dietary fiber in intestine by microbial fermentation (Bergman 1990), it can protect the intestinal barrier via up-regulating amount or promoting assembly of tight junction proteins, such as Claudin-1 (Reeves and Yip 1985; Werner et al. 2011), thereby bacteria and their metabolites are insulated from entering bloodstream so as to avoid an inflammatory response (Mortensen and Clausen 1996; Yan and Polk 2006). Meanwhile, SCFAs are the main source of energy for intestinal epithelial cells, and their decrease due to oral iron supplements will inhibit the growth of probiotics, impair the balance of intestinal strains, induce and even aggravate intestinal inflammation (Mcneil 1984; Ouwehand and Vaughan 1997; Avivigreen et al. 2002; Hofmanová et al. 2014; Bedford and Gong 2018; Feng et al. 2018; Pamela et al. 2014; Chang et al., 2011).

3. Measurement to reduce side effects of iron supplement

Since improper supplementation of iron can bring about a series of side effects (Heimbach et al. 2000; Millar et al. 2000), such as nausea, vomiting, diarrhea, and black stools (Tolkien et al. 2015; Steinbicker and Muckenthaler 2013), it is urgent to find effective measurement to reduce side effects and to recover intestinal flora based on full understanding the mechanisms of iron oxidative stress.

3.1. Alleviation of oxidative stress effects

It is well accepted that the severity of diarrhea in mice was dose dependent (Lin et al. 2018). The easier dissociation of Fe^{2+} from oral iron agents is, the more serious intestinal inflammation will occur (Werner et al. 2011). Because the oxidative stress is from excessive free radicals generated by ferrous ions through Fenton reaction and Haber-Weiss reaction, it can be theoretically alleviated by antioxidants via inhibiting the formation of free radicals and peroxides (Steinbicker and Muckenthaler 2013). Indeed, VE has been reported to alleviate the diarrhea caused by iron supplements via terminating the oxidization chain-reactions and preventing the formation of free radicals (Carrier et al. 2002). Another antioxidant, resveratrol, has been testified to mitigate myocardial oxidative damage resulting from high-iron diets (380 ppm) via altering iron homeostasis and lowering myocardial fibrosis (Das et al. 2015). Ferulic acid, possessing strong antioxidant capacity owning to its resonance stability, can neutralize the free radicals via transporting hydrogen atoms to phenolic hydroxyl groups (Kumar and Pruthi 2014). Qiao et al. further proved that ferulic acid could lessen liver damage and inflammation resulting from oxidative stress in a diet containing 2000 ppm of ferrocene (Qiao et al. 2016). Therefore, it is speculated that antioxidative substances can alleviate diarrhea originating from iron fortification, such as VC, VE, carotene, resveratrol-rich red wine, ferulic acid, and so on.

As mentioned above, the intrinsic factor for oral iron supplements to engender oxidative stress is free ferrous ion. Except for the redox equilibrium of antioxidants, another strategy is to isolate and to slowly release free iron ions. If ferrous citrate is encapsulated in liposome, this ferrous citrate liposome will release iron ions in a slow-release mode, which effectively reduces oxidative stress generating from free iron ions (Xu et al. 2014). Furthermore, the ferrous citrate liposomes are absorbed through lipid pathway instead of the conventional transferrin-mediated transport pathway, so they have good absorption efficiency without obvious side effects. (Xu et al. 2014). Sucrosomial® Iron, a kind of novel iron formulation in which ferric pyrophosphate is coated by a phospholipid bilayer plus a sucrester matrix (i.e., sucrosome), nearly has no adverse side effects due to the slow-release of free iron ions (Gómez-Ramírez et al. 2018). Besides, EDTA chelated iron can also prevent excessive free Fe^{2+} from producing excess free radicals and oxides, and it little changes the compositions of the intestinal microbes (Heimbach et al. 2000). Moreover, the stability of EDTA iron sodium (NaFeEDTA) is condition-dependent, NaFeEDTA converts into a tight structure in stomach, and it can be specifically absorbed in duodenum with preventing combination with phytic acid, it also promotes the absorption of dietary iron and zinc. If EDTA iron sodium serves as an oral iron supplement, intestinal inflammation such as diarrhea will be efficaciously relieved (Fairweather-Tait and Teucher 2002).

3.2. Recovery of intestinal micro-ecological balance

The intestinal micro-ecological balance is key factor for us to keep healthy. Gareau et al. demonstrated that probiotics could prevent and cure diarrhea induced by inflammation

(Gareau et al. 2010; Ohland and MacNaughton 2010). Common probiotics include *Lactobacilli*, *Bifidobacteria*, etc., they can lower pH of colon, form biological barrier, restore the isolating and protecting functions of intestinal mucosal barrier (Allen et al. 1982; Ohland and MacNaughton 2010), as well as inhibit the invasion of pathogen (Gareau et al. 2010). Probiotics can also promote the production of immunoglobulin A in the intestine, supporting the intestinal tract to form a "biological barrier" and "immune barrier", further promote the healing of inflammatory bowel disease. Lin *et al* testified that the addition of 10^9 CFU/ml *Lactobacillus* and 5 g/kg inulin to 500 ppm of oral ferrous sulfate could alleviate diarrhea caused by iron fortification and effectively reduce mortality (Gareau et al. 2010). Because *Lactobacillus* can effectively inhibit pathogens to adhere to the intestinal lining, stimulate the expression of Mucin 2 (Muc2) gene, increase the secretion of Muc2, Muc3 and other related mucins (Sina et al. 2018; Leser and Mølbak 2009; Johansson et al. 2013; Johansson 2014) ; in which, Muc2 mucin is secreted by goblet cells and it can hinder the colonization of pathogens, its content is closely related to the severity of intestinal inflammation and diarrhea (Sina et al. 2018). Moreover, *Lactobacillus* can effectively restore intestinal flora (Ohland and MacNaughton 2010; Achamrah et al. 2016). *Bifidobacterium* is a probiotic that produces vitamins, enzymes, acetic acid, and lactic acid. It can compete with pathogens for adhesion sites or nutrient sources with promoting the host's immune ability, inactivating microbial toxins, and generating substances to inhibit or kill pathogens (Gareau et al. 2010).

Now phage therapy, *Firmicutes*, and *Saccharomyces cerevisiae* are also applied to restore the intestinal micro-ecological balance (Baumgart and Dignass 2002). Multi-probiotic therapies, e.g. Fecal Microbiota Transplantation (FMT), Bacterial Consortium Transplantation (BCT) and synbiotics (Probiotics and prebiotics) can aid intestine to restore functions (Stoltzfus et al. 2004; Baumgart and Dignass 2002; Gagliardi et al. 2018). Feng et al. pointed out that SCFAs could form a gut barrier in a sub-*in vitro* model (Feng and Stockwell 2018).

Moreover, rhamnogalacturonan (RGal) separated from the *Acmella oleracea* (L.) RK Jansen leaves was found to alleviate intestinal inflammation caused by dextran sulfate sodium (DSS) (Maria-Ferreira et al. 2018). RGal is able to protect colonic epithelium, increase the stability of mucosal intestinal cells and promote mucus secretion of goblet cells, and retain collagen homeostasis and improve cell proliferation. It is further proved that RGal has a repair function for intestinal barrier *in vitro* and *in vivo* (Maria-Ferreira et al. 2018). In addition, both Sulfated polysaccharide from sea cucumber (SCSP) and depolymerized SCSP (d-SCSP) positively regulate the intestinal flora, such as increasing microbial diversity, increasing SCFA-producing bacterial and sulfide-degrading bacteria, as well as reducing harmful bacteria. Furthermore, SCSP and d-SCSP can also effectively adjust body weight and intestinal tissue index (Zhu et al. 2018). Up till now, SCSP has been used as an intestinal flora manipulator via a molecular weight (Mw)-dependent manner to promote health.

Additionally, trefoil peptide, glucagon-like peptide and bombesin are also found to rebuild intestinal micro-ecological balance (Baumgart and Dignass 2002). For instance, bovine lactoferrin can chelate iron ions, which avoids the existence of free iron ions (Chen et al. 2016). Subsequently, the nutrient source of iron is decreased, the growth and propagation of some potential pathogens are efficaciously inhibited, such as *Enterococcus Faecium*, *Salmonella* and *Fusobacterium* (Tomita et al. 1991). As a result, the proportion of these harmful bacterial could be reduced, their adverse effects e.g. diarrhea and vomit would be repressed and even disappear.

4. Conclusion

Excessive irons in the gastrointestinal tract usually induce Fenton reaction and Haber-Weiss reaction, abundant ROS e.g. superoxide anion (O_2^-) and hydroxyl radicals (OH·) are produced. ROS attack C = C bonds of fatty acids on cell membrane with disrupting cell membrane, which may trigger a cell death pathway, ferrotosis. Moreover, ROS may cause dysfunction of mitochondria and ER, which may activate the apoptotic and necrosis proteins, such as caspase, PERK, IRE1, RIPK, etc. Thereby, intestinal epithelial cells will be damaged or even die in apoptosis or necrosis model, subsequently, intestinal inflammation occurs or worsen. Furthermore, excess irons enhance the adhesion, translocation, and invasiveness of related pathogens. An imbalanced intestinal micro-ecology appears *in vivo*, triggering a series of side-effects, such as diarrhea, abdominal pain, vomiting, etc. In order to reduce the side effects induced by excessive iron, antioxidants, including VE, VC, carotene, ferulic acid, resveratrol-rich red wine, and iron-chelation, can be used to counteract free radicals and alleviate the damage to cells. Besides, liposome is also applied to prepare the iron-liposome complexes so as to slowly release free iron ions as well as avoid the accumulation of excess free iron ions. The novel oral iron have been designed and prepared, e.g. ferrous citrate liposome, EDTA iron sodium, and sucrosome iron. In the future, the new oral iron may be in the form of liposomes or complex. Meantime, probiotics and prebiotics, BCT and FMT have been developed to restore intestinal microbial balance, rebuild the intestinal biological barrier and immune barrier, and cure intestinal inflammation caused by iron fortification. With full understanding of the mechanisms of iron side effects, more and more iron supplements with high safety performance will be prepared, and some efficacious intervention measures to maintain the micro-ecological health of gastrointestinal tract will be developed.

Disclosure statement

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ORCID

Yane Luo http://orcid.org/0000-0002-0273-1195

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