REDOX REPORT, 2017
VOL. 22, NO. 1, 1–9
http://dx.doi.org/10.1080/13510002.2016.1229893

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The effect of antioxidant supplementation on bacterial translocation after intestinal ischemia and reperfusion

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ABSTRACT

The intestine is highly sensitive to ischemia/reperfusion (I/R) injury. Intestinal I/R may cause local tissue injury and disruption of the intestinal mucosal barrier, allowing the passage of viable bacteria and endotoxins from the gastrointestinal lumen to distant organs. This phenomenon, known as bacterial translocation (BT), may lead to systemic disorders with high morbidity and mortality. Oxidative stress mediators such as reactive oxygen species, polymorphonuclear neutrophils and nitric oxide are believed to contribute to the intestinal I/R injury. Many antioxidants have shown protective effects against I/R injury of various organs. The present article provides an overview of studies investigating the effect of antioxidant supplementation on BT after intestinal I/R.

KEYWORDS

Bacterial translocation; intestinal ischemia; reperfusion injury; oxidative stress; reactive oxygen species; free radicals; antioxidants; NO toxicity

Introduction

The gastrointestinal tract is highly sensitive to ischemia–reperfusion (I/R) injury and even short periods of ischemia can induce substantial local tissue damage. Intestinal I/R injury is a potentially grave condition resulting from various medical or surgical diseases, such as acute mesenteric ischemia, intestinal obstruction, incarcerated hernia, hemorrhagic, traumatic or septic shock, thermal injury and certain surgical procedures including small intestinal transplantation, cardiopulmonary bypass or abdominal aortic aneurysm [1,2].

Bacterial translocation (BT) is defined as the passage of viable bacteria or endotoxins from the gastrointestinal lumen to extra-luminal tissues through the mucosal epithelium, such as the mesenteric lymph nodes (MLNs) and distant organs [3]. It has been suggested that intestinal I/R induces disruption of the intestinal mucosal barrier, allowing translocation of bacteria and endotoxins to proximal tissues or distal organs. This phenomenon may stimulate the activation of inflammatory mediators, and the development of systemic inflammatory response syndrome, sepsis and multiple organ dysfunction syndrome [4,5].

Although the exact mechanisms involved in the pathogenesis of intestinal I/R injury and BT remain obscure, it is generally believed that certain oxidative stress mediators, such as reactive oxygen species (ROS), polymorphonuclear neutrophils and nitric oxide (NO) play a crucial role [6]. Therefore, it is reasonable to assume that the use of antioxidants could be beneficial in this context. Moreover, several studies have shown reduced I/R injury of various organs secondary to the use of antioxidant factors [6,7]. The aim of this article is to present the available data regarding the effects of antioxidants on I/R-mediated BT.

Methods

A literature search was conducted using a systematic approach. The PubMed database was comprehensively searched for relevant articles, using 'intestine', 'ischemia', 'reperfusion', 'bacterial translocation' and 'antioxidant' as keywords. All articles not available in English and all *in vitro* studies were excluded, while cross-referencing was performed using the bibliographies from the articles obtained.

Assessment for relevance to the particular question resulted in 11 articles, which were selected for the final review (Table 1). The search did not reveal any relevant human trials and only experimental studies were included. There was heterogeneity among the included animal studies with respect to the method of intestinal I/R. Models of superior mesenteric artery (SMA) clamping were the most common, with diversity in the duration of ischemia and the subsequent reperfusion. Models of thermal injury and a model of heterotopic small bowel transplant also were included (Table 1). Because of the heterogeneity among the included studies, quantitative synthesis of the extracted data

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Table 1. Summary of experimental studies that investigate the effect of antioxidants on bacterial translocation after intestinal ischemia/reperfusion.

Study	Antioxidant – Route of administration	Method of I/R induction	Population	Outcome
Zhi-Yong *et al.* [11]	*Rubia yunnanensis* – IV	Partial occlusion of the SMA for 1 hour (venous blood was obtained immediately after the occlusion, 1, 2, 4 ,24, 48 and 72 hours after reperfusion)	40 dogs divided in four groups of 10: • Control • I/R • I/R + Amikacin • I/R + *Rubia yunnanensis*	There was no statistical proof of significant attenuation of BT by *Rubia yunnanensis*
Sakai *et al.* [14]	Plaunotol – PO	Occlusion of the SMA for 45 minutes (blood samples were obtained on day 3 and day 7 post-surgery)	24 male Sprague-Dawley rats divided in four groups of six: • Control • Preventive dose • Therapeutic dose • Full dose	Preventive and full dose of plaunotol attenuated the translocation of endotoxin, indicated by its blood levels 3 days post-surgery
Oztürk *et al.* [16]	Selenium – IP	Occlusion of the SMA for 30 minutes (tissue samples were collected 24 hours later)	32 male Sprague-Dawley rats divided into four groups of eight: • Sham • I/R • Laparotomy + selenium • I/R + selenium	Selenium supplementation prevented I/R-induced BT
Colak *et al.* [22]	Trapidil – IV	Occlusion of the SMA for 40 minutes (followed by reperfusion for 12 hours)	40 adult male Wistar rats divided into four groups of 10: • Sham • Sham + trapidil • I/R • I/R + trapidil	Treatment with trapidil (I/R + trapidil) resulted in markedly decreased incidence of BT compared to the I/R group
Azuara *et al.* [24]	L-NAME (NO synthesis inhibitor) – Route of administration not mentioned	Heterotopic small bowel transplantation with 3 hours of cold ischemia and 5 hours of reperfusion	28 male Sprague-Dawley rats divided into four groups of seven: • Sham • Bowel transplant • Bowel transplant + NO donor • Bowel transplant + NO synthesis inhibitor	Exogenous administration of NO protected the transplanted graft against I/R injury and BT. Inhibition of NO synthesis did not display this protective effect
Ocal *et al.* [30]	NAC – IP	Thermal injury (induced by exposing the shaved dorsal skin of rats to boiling water for 12 seconds, under anesthesia. Tissue sampling was performed 24 hours after the thermal injury)	32 Wistar rats divided into four groups of eight: • Sham • Burn • Pre-burn NAC injection • Post-burn NAC injection	NAC supplementation prevented BT in both pre-burn and post-burn groups
Sileri *et al.* [31]	Melatonin – IP	Occlusion of the SMA for 45 minutes (tissue samples were collected 24 hours after reperfusion)	22 male ACI rats divided into three groups: • Melatonin ($n = 8$) • Placebo ($n = 8$) • Sham-operated ($n = 6$)	Melatonin significantly reduced the incidence of BT to the peritoneal cavity, MLNs, spleen and liver
Karabeyoğlu *et al.* [38]	Ethyl pyruvate – IP	Thermal injury (induced by exposing the shaved dorsal skin of rats to boiling water for 12 seconds, under anesthesia. Tissue sampling was performed 24 hours after the thermal injury)	32 Wistar rats divided into four groups: • Sham • Sham + ethyl pyruvate • Burn • Burn + ethyl pyruvate	Ethyl pyruvate supplementation significantly reduced the incidence of BT to the MLNs and the spleen. The attenuation of BT to the liver was not significant

(*Continued*)

Table 1. Continued.

Study	Antioxidant – Route of administration	Method of I/R induction	Population	Outcome
Berber *et al.* [46]	Tempol – IV	Occlusion of the SMA for 60 minutes followed by reperfusion for 24 hours	30 male Wistar-albino rats divided into three groups of 10: • Sham • I/R • I/R + tempol	Tempol treatment significantly attenuated BT to the MLNs, liver and spleen
Ozkan *et al.* [48]	Resveratrol – IP	Occlusion of the SMA for 60 minutes followed by reperfusion for 60 minutes	32 female Wistar-albino rats divided into four groups of eight: • Sham • I/R • I/R + vehicle • I/R + resveratrol	Treatment with resveratrol reduced BT in MLNs, spleen and liver, compared to the other I/R groups
Sözen *et al.* [34]	Melatonin – IM	Occlusion of the SMA for 60 minutes followed by reperfusion for 2 hours	40 Wistar-albino rats divided into four groups of 10: • Sham • I/R • I/R + glutamine • I/R + melatonin	The results of blood and MLN cultures indicated significant attenuation of BT by melatonin treatment. PCR, used to detect *E. coli* genomic DNA in the extracted blood samples did not indicate this protective effect

BT: bacterial translocation; IM: intramuscular; IP: intraperitoneally; I/R: ischemia/reperfusion; IV: intravenous; L-NAME: NG-nitro-l-arginine methyl ester; MLNs: mesenteric lymph nodes; NAC: *N*-acetylcysteine; NO: nitric oxide; PO: per Os; SMA: superior mesenteric artery

was not feasible. Therefore, a qualitative approach was chosen in order to answer the review question.

Antioxidants

Rubia yunnanensis

Rubia yunnanensis Diels (Rubiaceae) is a perennial herb native to Yunnan province, China. Its roots have a long history of use in traditional Chinese medicine as a sedative and blood-activating drug for the treatment of vertigo, insomnia, tuberculosis, menoxenia, rheumatism, contusion, hematemesis, anemia and lipoma [8]. Phytochemical studies showed that rubiaceae-type bicyclic hexapeptides, anthraquinones and triterpenoids are the major types of chemical constituents of this plant [9,10], some of which are shown to possess antioxidant properties [10]. In 1992, Zhi-Yong *et al*. [11] investigated the effects of IV-administered *Rubia yunnanensis* in a canine model of intestinal I/R, caused by partial occlusion of the SMA for 1 hour. *Rubia yunnanensis* prevented the decrease of superoxide dismutase (SOD) levels and the elevation of malondialdehyde (MDA) levels in tissues and plasma and also prevented the development of organ injury. There was no statistical proof of significant attenuation of BT by *Rubia yunnanensis*.

Plaunotol

Plaunotol [(2E, 6Z, 10E)-7-hydroxymethyl-3,11,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol)] is an acyclic diterpene alcohol originally isolated from the plant *Croton sublyratus*, which is native to southeast Asia. Plaunotol has been used to treat gastritis and gastric ulcers in Japan [12]. It is suggested to possess antioxidant properties [12,13] and was proven to be effective against gastric injury induced by I/R [13]. In 2000, Sakai *et al*. [14] used *per os* (PO) plaunotol in a rat model of intestinal I/R, caused by SMA occlusion for 45 minutes. They showed that a preventive (30 mg/kg/day 1 week before surgery) and a full dose (30 mg/kg/day 1 week before surgery and 1 week after surgery) of plaunotol attenuated the translocation of endotoxin, indicated by its blood levels 3 days post-surgery. A therapeutic dose of plaunotol (30 mg/kg/day 1 week after surgery) did not display this effect.

Selenium

Selenium, an essential trace element involved in many physiological functions, is known to have an antioxidant effect as a critical cofactor for the function of the enzyme glutathione peroxidase, which is involved in the oxidation of glutathione [15]. It was shown that selenium can be effective in preventing I/R injury in the heart, lung and kidney [16]. Oztürk *et al*. [16] studied the effects of intraperitoneally (IP) administered selenium on intestinal fine morphology, lipid peroxidation and BT, using a rat model of intestinal I/R in which the SMA was occluded for 30 minutes. Selenium supplementation significantly reduced the I/R-induced intestinal injury ($P < 0.05$) and prevented I/R-induced

BT. Moreover, tissue MDA levels from the ileum specimens were significantly lower in the selenium-treated group compared to the I/R alone group ($P < 0.05$).

Trapidil

Trapidil (5-methyl-7-diethylamino-s-triazolopyrimidine) was originally developed as an antianginal drug, and is a phosphodiesterase and platelet-derived growth factor inhibitor. Its pharmacological properties include nitroglycerine-like vasodilation, inhibition of platelet aggregation via blockage of thromboxane A_2, facilitation of the biosynthesis of prostacyclin, reduction of lipid peroxidation and inhibition of TNF, IL-6 and IL-12 production [17,18]. Trapidil was proven effective in preventing tissue damage in animal models of I/R injury of various organs [19–21]. In 2003, an experimental study explored the possible effects of IV administration of trapidil in small intestine injury, oxidative stress and BT in a rat model of intestinal I/R. The SMA was occluded for 40 minutes and reperfusion lasted 12 hours. Trapidil administration resulted in a significantly lower incidence of BT in the liver, spleen, peritoneum and MLNs. It also resulted in significantly lower blood levels of MDA and in a significantly attenuated histological degree of tissue damage [22].

NO donor vs. NO synthesis inhibitor

NO is a short-lived reactive molecule that plays an important role in many physiological and pathophysiological processes. Nitric oxide is the product of NO synthase (NOS), which catalyzes the conversion of arginine and oxygen (O_2) into NO and citrulline. There are three isoforms of NOS, each encoded by a different gene. Two of these isoforms, endothelial NOS (eNOS) and neuronal NOS (nNOS), are expressed constitutively at low levels; these enzymes produce picomolar concentrations of NO. In contrast, iNOS is not expressed under normal conditions, but is induced in high levels during inflammation. Once iNOS is expressed, it produces NO in nanomolar to micromolar concentration. NO is an intermediate oxidation product of nitrogen; it has an unpaired electron and therefore is a free radical. NO is a weak oxidant, but when it combines with superoxide anion (O_2^-) to generate peroxynitrite ($OONO^-$), it becomes a potent oxidant [23].

The role of NO in I/R is still a matter of controversy. Inhibition of NO synthesis causes tissue dysfunction in certain models of I/R and provides benefit in others. Azuara *et al*. [24] investigated the effect of NO on apoptosis, cell necrosis and BT associated with heterotopic small bowel transplant. In this study, Sprague-Dawley rats underwent heterotopic small bowel transplants with 3 hours of cold ischemia and 5 hours of reperfusion. Animals were assigned to the following study groups: sham; bowel transplant (Trp); bowel transplant + NO donor (Trp + NONOS); bowel transplant + NO synthesis inhibitor (Trp + L-NAME). It was shown that exogenous administration of NO protected the transplanted graft against I/R injury and BT, but it stimulated apoptosis.

N-Acetylcysteine

N-Acetylcysteine (NAC) is the most widely used antioxidant in experimental and clinical models. NAC is converted, *in vivo*, to l-cysteine, which is used in the repletion process of intracellular glutathione [7]. NAC protects against I/R-induced tissue damage due to its ability to scavenge ROS and its anti-inflammatory effects such as suppression of cytokine expression/release, inhibition of adhesion molecule expression and inhibition of nuclear factor kappa B actions [25–28]. Use of NAC in animal models of I/R injury demonstrated positive results [25,29]. An experimental study [30] investigated the possible effect of IP-administered NAC on intestinal oxidative stress and BT after thermal injury. A rat model was used where thermal injury was induced by exposing the shaved dorsal skin of rats to boiling water for 12 seconds, under anesthesia. Tissue sampling was performed 24 hours after the thermal injury. NAC, either administered 15 minutes before or 2 hours after the burn injury, significantly decreased the level of lipid peroxidation and myeloperoxidase (MPO) activity in intestinal tissue and prevented the occurrence of burn-induced BT.

Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) is secreted in circadian rhythm from the pineal gland as an endogenous hormone. It is also synthesized and secreted in retina, salivary glands, liver, thyroid and intestine [31]. Tan *et al* [32], using an *in vitro* system, established melatonin as a free radical scavenger in 1993. Melatonin also performs its antioxidant activity by stimulating glutathione peroxidase activity, which metabolizes hydrogen peroxide to water [33], and was proven to be effective in attenuating I/R injury in the liver, lung and intestine [31]. Sileri *et al*. [31] explored the possible effect of IP-injected melatonin on BT, using a rat model of 45-minute SMA occlusion, in which sampling was performed 24 hours after reperfusion. Melatonin significantly reduced the incidence of BT to the peritoneal cavity, MLNs, spleen and liver. It also significantly reduced the degree of intestinal I/R injury. Melatonin was also proven to be effective in another rat model of intestinal I/R, induced by SMA occlusion for 60 minutes with a 2-hour reperfusion period. Melatonin, administered via the IM route, prevented intestinal tissue injury and significantly decreased the incidence of BT, as indicated by the

results of MLN and blood cultures ($P < 0.05$). Interestingly, the results of PCR, which was used to detect *E. coli* genomic DNA in the extracted blood samples, did not reveal any significant difference ($P > 0.05$) [34].

Ethyl pyruvate

Pyruvic acid ($CH_3COCOOH$) is the simplest of the alpha-keto acids, with a carboxylic acid and a ketone functional group. Pyruvate, the conjugate base ($CH_3COCOO-$), is a key intersection in several metabolic pathways and also was proven to be a potent ROS scavenger, directly neutralizing peroxides and peroxynitrite and also scavenging hydroxyl radicals [35–37]. However, the usefulness of pyruvate as a therapeutic agent is abrogated by its very poor stability in solution. To overcome this problem, ethyl pyruvate – a derivative of pyruvic acid – has been formulated in a calcium- and potassium-containing balanced salt solution named Ringer's ethyl pyruvate solution (REPS) [35]. It was shown that REPS is an effective anti-inflammatory agent and this solution can improve the outcome in a variety of animal models of critical illness, such as hemorrhagic shock, sepsis and I/R injury [46]. Karabeyoğlu *et al*. [38] investigated the effects of IP-administered ethyl pyruvate in a rat model of intestinal I/R caused by thermal injury. The shaved dorsal skin of rats was exposed to boiling water for 12 seconds, under anesthesia. Tissue sampling was performed 24 hours later. The results showed that ethyl pyruvate prevented BT to the MLNs and spleen, but not to the liver. Ethyl pyruvate also prevented MDA and MPO production in the intestine, following thermal injury.

Tempol

Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl), a stable piperidine nitroxide of low molecular weight (MW: 172), is a water-soluble analogue of the spin label TEMPO, which is widely employed in electron spin resonance spectroscopy [39]. Tempol permeates biological membranes and, as an antioxidative agent, it scavenges superoxide anions *in vitro* and may act as a genuine 'SOD-mimetic' [40,41]. Tempol also reduces the formation of hydroxyl radicals either by scavenging superoxide anions or by reducing the intracellular concentrations of Fe^{2+} and, hence, the formation of hydroxyl radicals via the Fenton or Haber–Weiss reactions [42–44]. Its possible protective role against I/R injury has been extensively studied. Tempol was proven to be effective in animal models of I/R of various organs such as the heart, intestine, kidney and brain [45]. Berber *et al*. [46] used a rat model of intestinal I/R in order to study the effects of IV-administered tempol on various parameters, including BT. The SMA was occluded for 60 minutes and tissue samples were collected 24 hours after reperfusion. Tempol treatment significantly attenuated intestinal mucosal injury, neutrophil accumulation, lipid peroxidation and BT to the MLNs, liver and spleen.

Resveratrol

Resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) is a natural phytoalexin that is found in dietary sources such as grapes, plums, peanuts and wine (especially red wine) [47]. A growing body of evidence indicates that resveratrol may play potential therapeutic roles in human health by its antioxidant, anti-inflammatory, antiaging, anti-atherogenic, antidiabetic, anti-carcinogenic and antiapoptotic properties [48]. Resveratrol modulates key enzymes regulating cell life including cyclooxygenases (COX-1, COX-2), iNOS, lipooxygenase, protein kinase C and others [49]. It is an effective scavenger of hydroxyl and superoxide radicals and exhibits a protective effect against lipid peroxidation in cell membranes and DNA damage caused by ROS [50].

Resveratrol displayed beneficial effects in numerous experimental studies of I/R. It was protective against I/R injury of the heart, kidney, brain, intestine, liver, lung, testicles, ovaries, retina, bladder and skeletal muscles [51]. In 2009, Ozkan *et al*. [48] explored the effect of IP-administered resveratrol in an experimental study of intestinal I/R. In this rat model, I/R was induced by occlusion of the SMA for 60 minutes, followed by a 60-minute reperfusion period. Resveratrol significantly ameliorated the intestinal injury, prevented the increase of MDA, MPO and NO levels, restored the SOD activity and attenuated BT to the MLN's, liver and spleen.

Discussion

Although many mediators are implicated in I/R injury, including complement, neutrophils and various vasoactive mediators, ROS may be the most pertinent agents in this process [52]. Indeed, several studies have identified oxygen-derived free radicals as the mediators of the reperfusion component of I/R injury [53–56]. They are generated by xanthine oxidase (XO), MPO, iNOS, arachidonic acid derivatives or transition metals such as univalent copper or divalent iron [57,58]. ROS are toxic molecules; unless their concentration is regulated, they can cause protein, membrane and DNA damage and ultimately cell death [59].

Intestinal I/R occurs when intestinal blood flow is temporarily interrupted. The tissue damage is primarily caused by re-entry of oxygen during the reperfusion period, rather than by ischemia itself; ischemia followed by reperfusion is more damaging than ischemia without reperfusion and is characterized by severe pathophysiological disturbances [60]. Xanthine/XO is the initial source of oxygen radicals that can directly

cause lipid peroxidation or elicit neutrophils to produce other oxygen radicals and thus promote tissue injury [61]. During the ischemic period, cellular ATP is catabolized to yield hypoxanthine [62]. The hypoxic stress also triggers the conversion of xanthine dehydrogenase (XDH) to the oxygen radical-producing XO. Adenosine triphosphate depletion results in loss of ATP-dependent ion channel regulation, producing passive ion shifts across cell membranes: K^+ and Mg^{2+} diffuse out, but Na^+, Ca^{2+} and H_2O diffuse in, causing cell swelling. Increased cellular Ca^{2+} activates a calcium-dependent protease, which cleaves XDH to XO. During reperfusion, molecular oxygen is reintroduced into the tissue and reacts with hypoxanthine and XO to produce a burst of oxygen free radicals, O_2^- and hydrogen peroxide (H_2O_2) [6,63]. Also, during ischemia, the fall of the intracellular pH decreases the stability of lysosomal membranes, activates lysosomal lytic enzymes, inhibits the binding of transitional metals such as iron to their carrier proteins (e.g. transferrin and ferritin) and results in increased free intracellular iron (Fe^{2+}), which accelerates *in vivo* free radical formation through the Haber–Weiss reaction [64].

Under physiological conditions, the damaging effects of O_2^- are prevented by SOD, which converts O_2^- to H_2O_2 [6]. Moreover, non-protein sulfhydryl containing compounds, especially GSH, are major constituents of cellular defense mechanisms against oxidative stress. GSH acts either directly or via glutathione peroxidase catalysis to scavenge the generated ROS [65]. After I/R, however, these natural defenses may have been attenuated [6]. O_2^- itself is a relatively low-energy radical, but it is responsible for production of the highly reactive and damaging hydroxyl radical ($OH^•$). Typically, $OH^•$ causes biological damage by stimulating the free chain reaction known as lipid peroxidation, in which $OH^•$ attacks the fatty acid side chains of the membrane phospholipids and causes organelle and cell disruption [6]. MDA, as the end product of fatty acid peroxidation, is a good indicator of oxidative injury and the degree of lipid peroxidation can be estimated by the amount of MDA in tissues [66].

Several studies demonstrated that burn injury and I/R injury are associated with elevated levels of MDA in different organs and tissues [67–69]. In seven of the 11 studies included in this review, the level of MDA in tissues or blood was measured and antioxidant treatment was proven to be effective in preventing its elevation [11,16,22,30,38,46,48]. In two studies [11,48], the SOD activity or level in the tissue was assessed, while in two others [30,46] the tissue levels of GSH were measured. I/R caused a significant decrease of the levels of these endogenous antioxidants, probably due to excess consumption during oxidative stress. Of note, antioxidant treatment attenuated this decrease.

Neutrophils play an important role in the course of I/R injury. The systemic activation of neutrophils after reperfusion appears to be secondary to mediators such as cytokines and ROS [70,71]. These activated neutrophils promote inflammation and further oxidative damage. It was reported that this vicious cycle of I/R injury, endothelial damage and neutrophil infiltration produces additional ROS [46]. MPO is a peroxidase enzyme, which in humans is encoded by the MPO gene and is most abundantly expressed in neutrophil granulocytes. It is a lysosomal protein stored in azurophilic granules of the neutrophil. MPO activity appears to be a reliable index of neutrophil accumulation in the inflamed tissue and can be used to measure the extent of inflammation in intestinal tissue subjected to I/R injury [48,72]. Four of the included studies [30,38,46,48] assessed neutrophil accumulation by estimating MPO activity. It was shown that treatment with antioxidants attenuated neutrophil accumulation and the inflammatory process in the ischemic–reperfused intestine.

Histological damage that occurs after intestinal I/R is characterized by neutrophil infiltration, segmental necrosis, shortening of villi, loss of intestinal villi, nuclear centralization and dimensional changes. In eight of the included studies, intestinal tissue specimens were evaluated histopathologically and use of antioxidants significantly reduced histological damage and ameliorated the deleterious effects of I/R on the tissue [11,16,22,24,31,34,46,48].

A normally functioning intestinal mucosa prevents the transfer of enteric bacteria and endotoxins into other organs and blood circulation. Gut barrier dysfunction is considered to be the major cause leading to infectious complications when patients suffer from impaired intestinal blood supply and insufficient nutrient support [52]. Physiologically, intestinal barrier function is composed of mucosal immunity and physical integrity. I/R injury impairs intestinal immune defense and induces dysfunction or destruction of endothelial and epithelial cells leading to breakdown of endothelial and epithelial integrity, thereby causing an increase in intestinal permeability and failure of the intestinal barrier [6,73]. I/R may also lead to bacterial overgrowth due to alterations of intestinal motor activity and this factor also contributes to the incidence of BT [62,74]. Antioxidant treatment displayed beneficial effects on intestinal barrier integrity and significantly reduced the incidence of BT in nine of the included studies [14,16,22,30,31,34,38,46,48].

Interestingly, in the NO donor vs. NO synthesis inhibitor study [24], the NO donor showed protective effects against tissue damage and BT, while the NO synthesis inhibitor did not display such effects. This result seems paradoxical, since NO is a free radical [23]. As mentioned above, the role of NO in I/R injury is still a matter of controversy. NO has a dichotomous

role as a cytotoxic and a cytoprotective molecule in intestinal I/R and inhibition of NO causes tissue dysfunction in certain models of I/R, whereas it provides benefit in others. The constitutive forms of NOS (nNOS and eNOS) are critical to normal physiology and inhibition of these enzymes causes tissue damage. NO has many beneficial effects in the intestine such as scavenging of oxygen free radicals, maintenance of normal vascular permeability, inhibition of smooth muscle proliferation, reduction of leukocyte adherence to the mesenteric endothelium, prevention of mast cell activation and inhibition of platelet aggregation [6]. In contrast, the induction of iNOS during inflammatory states produces large amounts of NO and peroxynitrite, leading to tissue injury. NO and peroxynitrite generate very stable nitrite (NO_2^-) and nitrate (NO_3^-) ions which accumulate in cells, leading to the formation of highly reactive intermediates, such as NO_2, N_2O_3 or NO. These intermediates cause nitration and nitrosation of important biological macromolecules such as DNA, RNA, proteins and lipids, thereby disrupting their function [75]. There is also evidence that NO and/or peroxynitrite can act on the mitochondria to inhibit cellular respiration as a trigger for apoptosis. Shedding of apoptotic enterocytes can result in a transient bare area through which BT can occur [76].

Lack of NO toxicity under normal conditions is due to the fact that eNOS and nNOS do not produce high concentrations of NO. Also, NO produced by eNOS rapidly diffuses into red blood cells, where it is converted into nitrate upon reaction with oxyhemoglobin. eNOS-derived NO was shown to be protective at the onset of I/R injury [77]. Moreover, selective inhibitors of iNOS were reported to be beneficial, whereas the use of nonselective inhibitors displayed deleterious effects in intestinal I/R injury [78]. However, eNOS may become dysfunctional during oxidative stress and eNOS itself can be a superoxide source [79]. Ozkan *et al*. [48] showed that the protective effect of resveratrol treatment against I/R injury and BT is accompanied by prevention of the increase of NO levels.

NO was reported to be involved in the protective process of ischemic preconditioning in rat models of intestinal transplantation [80,81]. In an NO donor vs. NO synthesis inhibitor study, NO acted as a cytoprotective molecule and exogenous administration of a NO donor protected the transplanted graft against I/R injury and BT, although it also stimulated apoptosis [24].

Conclusions

Antioxidant supplementation displays protective effects against intestinal I/R injury. In various animal models, use of antioxidants was reported to maintain intestinal barrier integrity, thus attenuating BT. Prospective human trials are necessary in order to determine the precise effects of antioxidants on intestinal I/R and BT and their potential role in clinical practice.

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