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Clinical Translation of Nitrite Therapy for Cardiovascular Diseases

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Abstract

The anion nitrite is an oxidative breakdown product of nitric oxide (NO) that has traditionally been viewed as a diagnostic marker of NO formation in biological systems. In this regard, nitrite has long been considered an inert oxidation product of NO metabolism. More recently, this view has changed with the discovery that nitrite represents a physiologically relevant storage reservoir of NO in blood and tissues that can readily be reduced to NO under pathological conditions. This has sparked a renewed interest in the biological role of nitrite and has led to an extensive amount of work investigating its therapeutic potential. As a result, nitrite therapy has now been shown to be cytoprotective in numerous animal models of disease. Given the very robust preclinical data regarding the cytoprotective effects of nitrite therapy it is very logical to consider the clinical translation of nitrite-based therapies. This article will review some of this preclinical data and will discuss the potential use of nitrite therapy as a therapeutic agent for the treatment of cardiovascular diseases including: ischemia-reperfusion injury (i.e. acute myocardial infarction and stroke), hypertension, angiogenesis, and as an adjunctive therapy for transplantation of various organs (i.e. liver and lung).

Introduction

The biological role of the anion nitrite (NO_2-) has undergone a significant transformation in recent years. For many decades nitrite, nitrite was considered an inert by-product of nitric oxide (NO) metabolism. At present, nitrite is recognized as a major storage form of NO in blood and tissues that can readily be reduced to NO and initiate cytoprotective signaling during pathological states. Systemic nitrite levels (0.3–1.0 μM in plasma and from 1–20 μM in tissue) [1,2] are derived from both endogenous and exogenous sources with as much as 70% of plasma nitrite originating from the oxidation of eNOS-derived NO [3] and 30% originating from dietary sources [4–6]. In general, the dietary component of plasma nitrite is derived from nitrate, as humans do not generally consume significant amounts of nitrite in their diet. Nitrate enters the stomach and then circulates in the blood and is converted into

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Conflict of Interest

D.J.L. is a participant on a pending U.S. patent, filed on October 14, 2003 through NIH (patent no. 60/511, 244), regarding the use of sodium nitrite in cardiovascular disease. D.J.L. is also a participant of a pending U.S. patent filed on November 15, 2007 (patent no. 61/003150) regarding the use of nitrite salts in chronic ischemia.

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nitrite by commensal bacteria in the mouth and gastrointestinal tract. Both sources of nitrite are important for maintaining steady-state nitrite levels in the plasma and tissues [7].

Although historically, nitrite was generally considered to be an inactive NO metabolite, there were early indications that nitrite could be reduced back to NO under certain conditions. For example, Furchgott noted that acidified solutions of sodium nitrite surprisingly produced robust transient relaxations of the rabbit aorta much like solutions of NO gas [8]. This led investigators to use acidified sodium nitrite as a simple NO donor to test vascular smooth muscle function in isolated blood vessels. In terms of cytoprotection, it was reported in 1990 that the infusion of acidified sodium nitrite (12.5 – 50 mmol/kg/hr) during myocardial ischemia significantly reduced myocardial cell death in cats [9]. Perhaps it was the similarities of acidified nitrite to solutions of NO gas and other NO donors, which precluded nitrite from being studied further as a potential source of NO. As such, nitrite remained designated an inert metabolite of NO until several years ago [10]. The paradigm shift in nitrite physiology can be attributed to the findings of several key studies. The first set of studies demonstrated that NO formation increased during myocardial ischemia independently of enzymatic activity [11]. In these studies, experiments using electron paramagnetic resonance (EPR) spectroscopy and chemiluminescence demonstrated that the generation and accumulation of NO from nitrite increases 100-fold under the acidic and highly reduced conditions of the ischemic myocardium [11,12]. The next set of studies demonstrated that the exogenous administration of nitrite could limit the extent of ischemia-reperfusion injury. Webb and colleagues [13] first reported using a Langendorff isolated heart model that the infusion of nitrite (10 and 100 μM) prior to ischemia reduced infarct size and was associated with comparable improvements in recovery of left ventricular function. Duranski et al [14] reported that nitrite was cytoprotective in *in vivo* mouse models of myocardial and hepatic ischemia-reperfusion injury. In this study, solutions of sodium nitrite (2.4 – 960 nM) were administered during myocardial or hepatic ischemia. In the hepatic ischemia-reperfusion model, nitrite exerted profound dose-dependent protective effects on cellular necrosis and apoptosis, with highly significant protective effects observed at near-physiological nitrite concentrations (48 nM). In the myocardial ischemia-reperfusion model, nitrite reduced cardiac infarct size at 24 hours following reperfusion by 67%. Consistent with hypoxia-dependent nitrite bioactivation, nitrite was reduced to NO, S-nitrosothiols, N-nitros-amines, and iron-nitrosylated heme proteins within 1–30 minutes of reperfusion. Nitrite therapy has now been extensively studied in a variety of *in vitro* and *in vivo* animal models and has proven to be a highly effective means to reduce ischemia-reperfusion injury [15–20]. In terms of mechanisms of action, the cytoprotective effects of nitrite therapy have not been fully elucidated. However, it has been universally shown that nitrite-mediated protection is independent of eNOS and heme oxygenase-1 enzyme activities [14] and completely dependent on NO generation [13,14,21]. Therefore, the cytoprotective effects of nitrite therapy are likely similar to those attributed to NO.

There is no doubt that the field of nitrite biology and chemistry is an exciting area of research that continues to challenge the way we view the role that this small molecule plays in NO biology [10]. A direct result of our improved understanding of the biochemical conversion of nitrite to NO under both physiological and pathophysiological conditions has been a growing interest for the use of nitrite therapy in the clinic. This article will review the potential use of nitrite therapy as a therapeutic agent for the treatment of several clinical conditions.

Clinical Translation of Nitrite Therapy

NO has been extensively studied in the setting of ischemia-reperfusion injury [22,23]. Previous experimental studies clearly demonstrate that the deficiency of eNOS exacerbates

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I/R injury [24,25], whereas the overexpression of eNOS [26,27], the administration of NO donors [28–32] and inhaled NO gas therapy [33] are all cytoprotective [34]. In contrast, there have been some studies reporting negative effects of NO [35–37]. A review of the literature which investigated the role of NO in modulating the severity of ischemia-reperfusion injury in the non-preconditioned myocardium spanning the period from 1991 to 2001 found that 73% of the studies reported that NO (endogenous or exogenous) was cardioprotective, whereas 12% reported that NO was detrimental [34]. This very comprehensive analysis along with a more recent review [23] revealed that the discrepancies between these two opposing findings can be explained by the dose of NO investigated, as it was found that physiological levels (i.e., nanomolar) of NO promote cytoprotection and suprapharmacological levels (i.e., high micromolar and millimolar) mediate cellular necrosis and apoptosis.

There have also been inconsistent outcomes regarding the use of NO-based therapies in the clinical setting. For example, the ISIS-4 trial [38] reported that routine mononitrate therapy did not reduce death following acute MI, whereas inhaled NO has shown efficacy in certain clinical situations [39,40]. Additionally, there are several limitations to the currently used NO-based therapies that preclude its widespread use. First, inhaled NO therapy is rather expensive and requires a technically complex delivery system [41]. This limits its use to specialized controlled conditions and severely lessens the chance that this therapy will be available in most hospitals. Second, inhaled NO therapy and NO donor therapy have the potential to cause unwanted side effects, since both therapies rely on systemic delivery. For example, the class of NO donors known as the NONates release NO over a period of time depending on the half-life of the drug. Since, NO is continually released as soon as the drug enters the blood stream, there is no way of ensuring that the NO is released only at the site of injury. As a result, higher concentrations of the drug have to be administered to achieve the desirable therapeutic effects. This in turn can result in unwanted systemic side effects, such as hypotension or NO-mediated cytotoxicity. Alternative means to effectively deliver NO to the site of injury are thus necessary to achieve the therapeutic potential of NO-based therapies.

Based on work conducted over the past decade, it appears that nitrite therapy may be the solution to the current limitations of NO-based therapies. First of all, nitrite is a highly stable molecule that can be transported in the circulation and stored in tissues [2,42,43]. Second, nitrite releases NO under conditions that exist in injured tissue (ischemia, hypoxia, or low pH) [33,43–46], which allows nitrite to preferentially target injured tissue and reduce the risk of systemic hypotension and other unwanted side effects. This is probably the most important aspect of nitrite therapy and what distinguishes it from other therapeutic strategies that involve NO. Third, nitrite can be given through several different routes of administration including: oral administration, topical administration, intravenous administration, intraperitoneal administration and in an aerosolized form directly to the lungs and pulmonary circulation. The diversity in which nitrite can be administered certainly provides options when designing therapeutic strategies to combat clinical conditions and allows for one to tailor the treatment with the condition. For example, the administration of aerosolized nitrite may be the most effective strategy to combat pulmonary hypertension, whereas an intravenous administration of nitrite would be more effective for the treatment of acute myocardial infarction. It is also conceivable that novel nitrite formulations such as transdermal or subcutaneous preparations could be developed to promote local angiogenesis and wound healing in patients with impaired circulation such as diabetics [10]. Finally, since nitrite has been used for many years as part of the cyanide antidote kit in humans there is a wealth of clinical data to support the safety of very high doses of sodium nitrite in critically ill patients. No such data exists for NO gas and NO donors.

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Given the very robust preclinical data regarding the cytoprotective and vasodilatory [5,47–49] effects, of nitrite therapy it is very logical to consider the clinical translation of nitrite-based therapies for ischemia-reperfusion injury (i.e. acute myocardial infarction and stroke), hypertension, angiogenesis, and as an adjunctive therapy for transplantation of various organs (i.e. liver and lung). At present there are 11 clinical studies either underway or recently completed (ClinicalTrials.gov) to investigate the potential therapeutic actions of nitrite therapy in persons with acute myocardial infarction, cerebral vasospasm, pulmonary hypertension, sickle cell disease, and other disease states.

Nitrite Therapy and Stroke

A stroke is defined as the sudden loss of brain function due to the loss of blood supply to the brain. There are two types of strokes: ischemic or hemorrhagic. An ischemic stroke represents about 80% of all strokes and usually occurs when the blood supply to the brain is interrupted by a blood clot. A hemorrhagic stroke occurs when blood accumulates in the brain, usually when an aneurysm burst. Current therapeutic strategies aimed at alleviating injury following a stroke remain scarce. As such, stroke still remains a major cause of mortality and disability worldwide and remains a major socioeconomic burden [50].

Thrombolysis using recombinant tissue plasminogen activator (rtPA) remains the only treatment strategy for an acute ischemia stroke. Since intravenous rtPA increases the risk for hemorrhagic transformation, it is only used during the first 3 hours after the onset of an ischemic stroke [51]. This is an extremely tight therapeutic window given that most patients do not present within the first 3 hours of symptoms or that a physician is not certain when a stroke began. As a result, rtPA is administered to a very small number of patients. Therefore, in reality there is not an effective treatment for an ischemic stroke. Moreover, there are not any effective treatments for hemorrhagic stroke. Given this paucity of effective therapeutic treatments for stroke, researchers have spent a great deal of effort investigating potential therapeutic interventions. Recently, nitrite has been reported to provide protection in experimental models of stroke.

Jung and colleagues [17] investigated the therapeutic potential of nitrite in a rat model of middle cerebral artery occlusion. In this study, solutions of nitrite were infused intravenously at the time of reperfusion. The authors found that nitrite reduced infarction volume and enhanced local cerebral blood flow and functional recovery. More recently, this group also demonstrated that nitrite treatment could still provide profound neuroprotection when its administration was delayed to 3 hour of reperfusion [52]. In contrast to these two studies, Schatlo and colleagues [53] reported that nitrite in combination with rtPA did not provide any additional protection against middle cerebral artery occlusion over rtPA alone. There are several differences between the studies, which could account for the different outcomes. The first difference is the dose of nitrite and the administration of protocol. In the studies from Jung and colleagues, nitrite was administered at a dose of 480 nm as a 1-minute bolus. In the study from Schatlo and colleagues, nitrite was administered either at a dose of 7.5 μmol/L or 0.500 μmol/L as a 50-minute infusion. The low dose in the Schatlo study is the same as the dose used in the Jung studies, but the duration of administration is different. It is conceivable that the final dose administered to the rats in the Schatlo study was on the high side and could have resulted in the lack of protection. This would be consistent with the dose response curve reported by Duranski et al [14] and Jung et al [17]. There were also differences in the duration of ischemia. The studies by Jung and colleagues used 90 minutes of ischemia whereas the Schatlo study investigated 6 hours and 2 hours of ischemia. Finally, Schatlo administered nitrite with rtPA whereas Jung did not. Given the different findings of these studies, more work is definitely needed to evaluate the use of nitrite as a treatment for cerebral ischemia and the interaction of nitrite with rtPA.

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Subarachnoid hemorrhage (SAH) is a common and frequently devastating condition, accounting for ~ 5% of all strokes and affecting as many as 30,000 Americans each year [54]. A major event associated with SAH is cerebral vasospasm, which is the delayed narrowing of large-capacitance arteries at the base of the brain after SAH. Vasospasm is often associated with radiographic or cerebral blood flow evidence of diminished perfusion in the distal territory of the affected artery [55]. In about one half of cases, vasospasm is manifested by the occurrence of a delayed neurological ischemic deficit, which with equal likelihood may resolve or progress to cerebral infarction [56]. Despite major advances in surgical techniques, radiology, and anesthesiology, the mortality and morbidity rates after spontaneous SAH have not changed in recent years. In the past, research has concentrated primarily on vasospasm and its sequela, in an attempt to combat the high morbidity and mortality associated with SAH. To date, this has not resulted in a definitive treatment modality to prevent or ameliorate brain injury after SAH [57]. Pluta et al [19] reported that infusions of nitrite prevented delayed cerebral vasospasm in a non-human primate model of SAH. Currently, a clinical trial has just begun to recruit patients in an effort to determine if nitrite therapy can prevent cerebral vasospasm. The study is designed to examine the safety of a 14-day infusion of sodium nitrite, and to study the pharmacokinetics of nitrite, during a 14-day infusion in patients with ruptured cerebral aneurysms. The results of this ongoing clinical trial will provide very important insights into the potential clinical benefits of nitrite therapy in the setting of cerebral vasospasm and will certainly set the stage for the design of additional clinical trials aimed at evaluating the neuroprotective effects of nitrite.

Nitrite Therapy and Myocardial Ischemia

Acute myocardial infarction resulting from myocardial ischemia remains the number one cause of morbidity and mortality in the United States and is responsible for approximately 220,000 or 15% of the nearly 1.4 millions deaths related to cardiovascular disease. It is estimated that 1.1 million Americans will have a new or recurrent myocardial infarction this year [58]. Among patients that survive an acute myocardial infarction, the major determinant of the long-term prognosis is the amount of myocardium that is destroyed as a result of ischemic injury. Therefore, successful reperfusion of an occluded coronary artery leading to complete and sustained blood flow is associated with an optimal outcome, whereas a delay in restoring blood flow is associated with adverse effects [59]. Over the past several decades, significant advances have been made in therapeutic interventions designed to reperfuse an occluded artery. However, myocardial infarction still remains a major socioeconomic crisis in the industrialized world. A key factor in this discrepancy is that the effects of reperfusion are complex and paradoxically include deleterious effects. Therefore, adjunct pharmacotherapies designed to coincide with reperfusion are needed to decrease the extent of reperfusion injury. Despite extensive research efforts, few if any of the successful experimental agents have shown clinical efficacy.

As mentioned above, nitrite therapy has been shown to provide cardioprotection in *in vitro* and *in vivo* models of myocardial ischemia-reperfusion injury. These experimental studies have provided important insights into the cardioprotective effects of nitrite therapy and have demonstrated nitrite therapy to be equally effective when it is administered before, during or after ischemia through either systemic or oral administration [21]. So, the obvious question becomes, is nitrite ready for the clinic as a treatment for myocardial ischemia? It seems likely based on the promising results of a recent experimental study by Gonzalez et al [60], investigating the effects of a low dose of intravenous nitrite therapy, would enhance the efficacy of reperfusion therapy for acute myocardial infarction in a large animal model compatible with typical delays from onset of chest pain to emergent intervention. The authors investigated an *in vivo* canine model with a protocol of 2 hours of coronary artery ischemia followed by 6 hours of reperfusion. Nitrite therapy (0.20 μmol/min per kilogram)

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was administered either during the last 60 minutes of ischemia or during the last 5 minute of ischemia. They found that nitrite therapy limited myocardial infarction and apoptosis. Importantly, the mechanism of myocardial protection was found to be independent of the time/ischemia severity integral because the group infused with nitrite during the last 5 minute of ischemia experienced a reduction in infarct size and apoptosis almost to a similar degree as the 60-minute infusion group. This suggests that infusion of nitrite could be initiated prior to percutaneous coronary intervention [60]. Currently, a clinical trial has just been initiated in an effort to determine if the intravenous infusion of sodium nitrite safely prevents ischemia-reperfusion injury in subjects with acute myocardial infarction resulting in improved left ventricular function. The results of this clinical trial will determine the efficacy of nitrite therapy in the setting of acute myocardial infarction and will certainly open the door for future trials involving nitrite therapy and cardiovascular disease.

Another condition associated with cardiovascular disease in which nitrite therapy may provide protection is heart failure. Heart failure is a heterogeneous syndrome that can result from a number of common disease stimuli, including, but not limited to long-standing hypertension, myocardial infarction or ischemia associated with coronary artery disease [61,62]. The prevalence of heart failure has increased dramatically as modern therapies have reduced the in-hospital mortality of acute myocardial infarction [62]. In the United States, it has become the most common discharge diagnosis in patients aged 65 years or older and the primary cause of readmission within 60 days of discharge [63]. Current treatments for heart failure are woefully inadequate, and the availability of hearts for transplantation is severely limited [62]. Therefore, adjunct pharmacotherapies designed to coincide with the standard means of care are needed to decrease the extent of injury leading to the development of heart failure. The diversity in which nitrite can be administered makes it a prime candidate for the treatment of heart failure. A treatment strategy could consist of nitrite therapy being initiated at the time of percutaneous coronary intervention via an intravenous administration and then continued daily through an oral administration. Given the lack of data regarding the use of nitrite in heart failure, more experimental work in this area is warranted. In particular it will be important to determine if the environment of the failing heart is conducive to reducing nitrite into NO.

Nitrite Therapy and Hypertension

Nitrite has been reported to induce vasodilatation in several animal models [20,49,64] and in healthy human volunteers [47,65]. These vasodilatory effects may be of therapeutic potential for several clinical situations such as SAH, myocardial ischemia, and hypertension. Particularly, nitrite therapy has been considered a candidate for the treatment of primary pulmonary hypertension of the newborn (PPHN). PPHN is a condition that is associated with a high pulmonary vascular resistance and extremely low systemic oxygenation. Low dose inhaled NO therapy reduces the need for extracorporeal membrane oxygenation and reduces the occurrence of chronic lung disease in neonates [39,40]. Despite these encouraging results regarding treatment of persistent pulmonary hypertension of the newborn with inhaled NO, the therapy does have several significant limitations, such as considerable cost, technical difficulties involved in adapting NO delivery systems for neonatal transport and the lack of availability in small community hospitals and developing countries [41]. Therefore, alternative NO-based therapies are highly desirable. Hunter et al [41] investigated if nitrite therapy could be a viable alternative to inhaled NO therapy. Using a newborn lamb model of hypoxic-induced pulmonary hypertension, the authors found that inhaled nitrite elicited a rapid and sustained reduction in hypoxia-induced pulmonary hypertension, with a magnitude approaching that of the effects of 20 p.p.m. NO gas inhalation. Notably, from a therapeutic standpoint, short-term delivery of nitrite dissolved in saline through nebulization produced selective, sustained pulmonary vasodilation with no

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clinically significant increase in blood methemoglobin levels [41]. Experimental evidence also indicates that nitrite therapy for the treatment of pulmonary hypertension may not be limited to the newborn and may not be limited to an inhaled route as there is data to suggest that intravenous nitrite therapy can reduce pulmonary hypertension in an adult model of acute pulmonary thromboembolism [66]. These data support the concept that nitrite is a vasodilator acting through conversion to NO and support the notion that it may be a potential therapy for pulmonary hypertension. Furthermore, nitrite therapy is relatively inexpensive and the flexibility of its route of administration alleviates the need for a complex delivery system like the one required for inhaled NO therapy, which makes nitrite therapy available to small communities hospitals and developing countries. Currently the National Institutes of Health Clinic Center is conducting a phase I clinical trial to examine and test healthy volunteers and patients with pulmonary hypertension to try to learn more about the disease and find better ways to detect, treat, and, if possible, slow progression. One of the goals of this study is to test the effectiveness of aerosolized nitrite to alter lung and heart pressures of patients with pulmonary hypertension. Therefore, the results of this study will be the first step in determining the feasibility of such an approach.

There has also been a growing interest in recent years regarding the contribution of dietary nitrate/nitrite in the development and treatment of systemic hypertension. As noted above, nitrate accounts for the majority of nitrite derived from the diet. Nitrate enters the stomach and then circulates in the blood and is converted into nitrite by commensal bacteria in the mouth and gastrointestinal tract. Supplementation of sodium nitrate (0.1 mmol/kg/d) to healthy volunteers over a period of 3 days reduced diastolic blood pressure by 3.7 mm Hg but did not affect systolic blood pressure [67]. Webb et al [68] have also reported that dietary nitrate consumption can lower blood pressure. In this study, the mean arterial blood pressure of health volunteers reach a maximal reduction of 8 mm Hg at 3 hours after the consumption of 500 mL of beetroot juice. Although these studies indicate that nitrite, via its conversion from nitrate, can reduce blood pressure in healthy, non-hypertensive human subjects, in models of hypertension are warranted to determine if nitrite/nitrate therapy can lower blood pressure to the same extent as the current standard of care (i.e., angiotensin-converting enzyme inhibitors and angiotensin-II receptor blockers) treatment.

Nitrite Therapy and Transplantation

Nitrite therapy has proven to be cytoprotective in various models of ischemia-reperfusion injury [18] and has been shown to be an effective preconditioning agent [21]. Preconditioning strategies have always proven to be effective in experimental models but the clinical relevance of such strategies has always been called into question. As a result, the administration of nitrite during and after ischemia has garnered the most attention in the design of clinical trials, but the preconditioning effects of nitrite could prove to be just as important in certain clinical situations, such as organ transplantation. A major cause of graft failure in both liver and lung transplantation is ischemia-reperfusion injury [28,69–71]. For instance, the damage to the liver caused by the ischemia-reperfusion injury represents a continuum of processes [72] that may produce profound hepatocellular injury and ultimately result in morbidity and mortality [73]. Liver transplants have been on the rise for the last 15 years and a noted increase in demand and shortage of supply has forced the consideration of cadaveric or steatotic graphs [74], which have a higher susceptibility to ischemia-reperfusion injury and a much higher risk of primary nonfunction and mortality [75]. Therefore, minimizing the adverse effects of hepatic ischemia-reperfusion injury could increase the number of patients that may undergo a successful transplantation and could reduce the hospital stay for patients [76]. However, at present there is no treatment plan or strategy available to prevent hepatic ischemia-reperfusion injury [72]. The same scenario appears to exist for lung transplantation, as ischemia-reperfusion injury also continues to be

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a common and substantive cause of morbidity and mortality. Ischemia-reperfusion injury usually presents with the immediate impairment in lung function after transplantation accompanied by rapid development of pulmonary edema, increased pulmonary vascular resistance, and decreased airway compliance [69]. Treatment strategies for lung transplant patients that develop ischemia-reperfusion injury consist primarily of maintaining oxygenation and lung function.

Recently, there has been an interest in using NO-based therapies in organ transplantation. In 1996, Date and colleagues [77] reported that inhaled NO improved oxygenation and decreased pulmonary artery pressure without systemic circulatory effects in a small group of patients with severe allograft dysfunction. More recently, Yerebakan et al [78] also reported the beneficial effects of inhaled NO therapy in patients undergoing lung transplant. The same beneficial effects of inhaled NO therapy have also been reported for liver transplantation. In a recent prospective, blinded, placebo-controlled study, Lang and colleagues [79] evaluated the effects inhaled NO on patients undergoing orthotopic liver transplantation. In this study, patients were randomized to receive either placebo or inhaled NO therapy during the operative period only. When results were adjusted for cold ischemia time and sex, inhaled NO significantly decreased hospital length of stay, circulating liver enzyme levels and coagulation times, indicating that inhaled NO therapy improved the rate at which liver function was restored after transplantation. These clinical studies clearly demonstrate the efficacy of using NO-based therapies in the setting of organ transplantation. However, the same concerns regarding the use of inhaled therapy as mentioned for pulmonary hypertension still exists. Therefore, nitrite therapy may be a viable alternative to inhaled NO therapy. Recipient patients could be treated with nitrite 24 hours before the procedure and the donor organ could also be infused with nitrite. The stored nitrite could then potentially create an environment that reduces rejection of the organ and promotes a successful transplantation. Alternatively or collectively, nitrite could be administered intravenously at the time of transplantation and then during the recovery period as needed. Of course data demonstrating the effectiveness of such a strategy is needed before clinical trials can begin.

Nitrite Therapy and Angiogenesis

The treatment of peripheral vascular disease and chronic tissue ischemic states with therapies designed to induce angiogenesis remain elusive. To date, many factors and agents have been reported to induced angiogenesis in experimental models, but proangiogenic clinical trials have all failed [80]. Therefore, new therapeutic strategies to induce therapeutic angiogenesis are needed. NO has been reported to play a role in mediating vascular endothelial growth factor (VEGF)-mediated angiogenesis [81,82], suggesting that NO donor therapy would be very beneficial for therapeutic angiogenesis. Recently, Kumar and colleagues [83] were the first to demonstrate that chronic sodium nitrite therapy promotes vascular angiogenesis in a murine model system of hind-limb ischemia. This study very clearly demonstrates that nitrite administered intravenously restores ischemic hind-limb blood flow, stimulates endothelial cell proliferation, and stimulates angiogenesis in an NO-dependent manner. This study, therefore, provides strong support that nitrite promotes vasculogenesis, suggesting that nitrite therapy could be a potential therapy for peripheral artery disease as well as a therapy for chronic ischemic states such as myocardial ischemia and congestive heart failure.

Summary

When one looks back on the history of nitrite, it is apparent that this small molecule has come a long way in recent years. Not too long ago nitrite was considered an inert by-product

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of NO metabolism. Now it is recognized as a major storage form of NO in blood and tissues that can be readily reduced to NO under certain pathological conditions. This unique characteristic is perhaps what distinguishes nitrite from other NO-based therapies. Couple this with the multiple ways in which nitrite can be administered and it is easy to see why nitrite has garnered consideration as a therapeutic agent for the treatment of a variety of clinical conditions. As noted, there are 4 clinical studies currently underway to investigate the potential therapeutic actions of nitrite therapy in persons with acute myocardial infarction, cerebral vasospasm, and pulmonary hypertension. The results of these ongoing trials will provide very important insights into the potential clinical benefits of nitrite therapy and will set the stage for the design of additional clinical trials aimed at evaluating the cytoprotective effects of nitrite. Researchers will also continue to explore new areas in which nitrite therapy may be a viable therapeutic option and will also continue to expand on those areas in which nitrite therapy has shown promise.

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