Review Article

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Clinical application of nitric oxide in ischemia and reperfusion injury: A literature review

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Abstract:

Ischemia–reperfusion injury (IRI) is a series of multifactorial cellular events that lead to increased cellular dysfunction after the restoration of oxygen delivery to hypoxic tissue, which can result in acute heart failure and cerebral dysfunction. This injury is severe and would lead to significant morbidity and mortality and poses an important therapeutic challenge for physicians. Nitric oxide (NO) minimizes the deleterious effects of IRI on cells. NO donors, such as organic nitrates and sodium nitroprusside, are used systematically to treat heart failure, angina, and pulmonary hypertension. Inhaled NO gas was approved by the FDA in 1999 to treat hypoxic newborns, and its beneficial ameliorations reach outside the realm of lung disease. This review will summarize the clinical application of NO in IRI.

Keywords:

Inhaled nitric oxide, ischemic stroke, myocardial infarction, nitric oxide donor

Introduction

Historical perspective of nitric oxide

During the 1970s, it was recognized that endothelium causes vasodilation by releasing a factor that relaxes vascular smooth muscle cells.[1] At the time, the factor's chemical structure was unknown, so it was named endothelium-derived relaxing factor, but it was eventually identified as the colorless and odorless gas, nitric oxide (NO).[2,3] Since then, NO has been gradually recognized as a gas signaling molecule, and its mechanisms of action in the laboratory animals and humans have been extensively researched.

Mechanisms of nitric oxide in ischemia and reperfusion injury

Ischemia–reperfusion injury (IRI) can result in organ injury and failure through a complicated series of events due to inflammation mediated by intracellular injury. Ischemia induces anoxic injury, resulting in the loss of adenosine triphosphate production. As a result, cells fail to sustain their homeostatic functions, leading to intracellular alterations, such as increased membrane permeability and nonhomeostatic cytosolic pH. Reperfusion occurs with the re-establishment of blood flow along with molecular oxygen, following ischemia. However, during reperfusion, increased production of free oxygen radicals and decreased production of NO can lead to IRI. Previous studies demonstrate that organ injury can occur due to a reduction of NO, most commonly due to a reduction in endothelial NO synthase activity, during IRI.[4] In the setting of IRI, NO has been found to have various protective effects on inhibiting oxidative stress, leukocyte–endothelial adhesion, cytokine release, and apoptosis.[5] These neuroprotective effects have led to the clinical administration of inhaled NO or NO donor drugs to blunt IRI. This review will discuss the clinical use of NO in attenuating the impact of IRI [Figure 1].

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Clinical Trials of Nitric Oxide in Ischemic Stroke

Transdermal glyceryl trinitrate

A stroke most frequently occurs when the brain has perfusion defects from occluded blood vessels, ischemic stroke, or ruptured hemorrhagic stroke. Stroke has high morbidity and mortality. According to existing data, ischemic stroke and hemorrhagic stroke have a record number of more than one million new cases each year.[6] Furthermore, these new cases of stroke result in a heavy socioeconomic burden on the patient, his or her family, and society due to the substantial expenditure costs associated with stroke complication treatment at the hospital.[7] Current ischemic stroke therapies include both reperfusion and neuroprotection strategies, such as mechanical thrombectomy, stenting and angioplasty, surgical treatment (decompressive craniectomy and carotid endarterectomy), thrombolytic agents, neuroprotective drugs, and rehabilitation training.[8,9] As a potential treatment for managing acute stroke, glyceryl trinitrate (GTN), a NO donor, can provide hemodynamic stability, potential reperfusion, as well as neuroprotective effects.[10] Patients with acute and subacute stroke received GTN as a transdermal patch in three – phase II trials: Efficacy of NO in Stroke (ENOS), Rapid Intervention with GTN in Hypertensive stroke Trial (RIGHT), and RIGHT-2.[11-13] Across these trials, GTN lowered blood pressure (BP) (peripheral and central), 24-h BP, peak systolic BP (SBP), pulse pressure, and pulse pressure index; increased heart rate; improved vascular compliance; and did not alter cerebral blood flow and velocity or induce cerebral steal or increase intracranial pressure.[14-17]

Efficacy of Nitric Oxide in Stroke, Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial, and Multicenter Randomized trial of Acute Stroke treatment in the Ambulance with a nitroglycerin Patch

Efficacy of Nitric Oxide in Stroke

Efficacy of NO, with or without continuing antihypertensive treatment, for the management of high BP in acute stroke (ENOS) is a partial-factorial randomized controlled trial. ENOS enrolled 4,011 participants with acute stroke (within 48 h of onset) and elevated systolic BP (140–220 mmHg) and randomized these participants to transdermal GTN patch (5 mg) or no patch. Using the modified Ranking scale (MRS), there was no significant change in functional or secondary outcomes measured at day 90.[11] However, when transdermal GTN was given within 6 h, patients had improved functional outcomes and fewer deaths.[18] Furthermore, there was no increased reporting or incidence of serious adverse events, indicating that GTN is safe to administer.

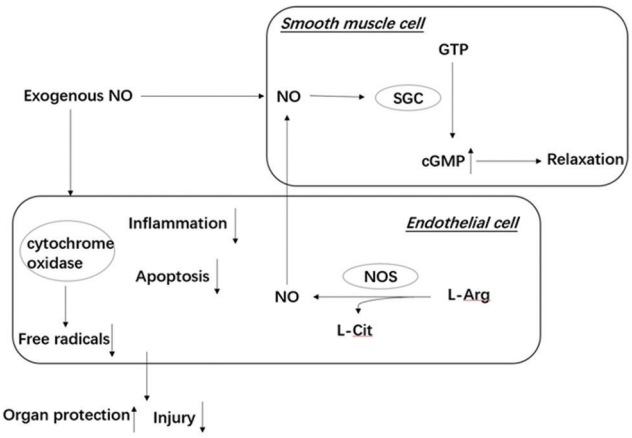

Figure 1: Possible mechanistic effects on the role of ischemic injury and exogenous nitric oxide in organ protection

Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial and Rapid Intervention with Glyceryl trinitrate in Hypertensive stroke Trial 2

Due to the improved functional outcomes from transdermal GTN as well as its physiologic properties, investigators sought to assess the feasibility of administering transdermal GTN by paramedics before hospitalization. The small ambulance-based RIGHT enrolled 41 patients who met certain stroke criteria and randomized these participants to receive or not receive a transdermal GTN patch (5 mg). They found that administration of transdermal GTN (5 mg) was safe in ultra-acute stroke. Furthermore, when paramedics administered the patch in the prehospital setting within 4 h of ictus, there was an improvement in functional outcomes measured by the MRS at day 90.[12] However, they acknowledged that the small size of the study limited any conclusive interpretations of functional the trial. As a result, the investigators of RIGHT launched RIGHT-2 to confirm whether GTN is efficacious when given early in patients with acute stroke. They enrolled 1,149 patients with presumed stroke and administered a transdermal 5-mg GTN patch or sham within 4 h of onset. Unfortunately, in an article published in The *Lancet* in 2019, the results from RIGHT-2 demonstrated that administration of a transdermal GTN in the prehospital setting for ultra-acute stroke did not alter the functional outcomes.[13]

Multicenter Randomized trial of Acute Stroke treatment in the Ambulance with a nitroglycerin Patch

One year before the publication of RIGHT-2 results, The Multicenter Randomized trial of Acute Stroke treatment in the Ambulance with a nitroglycerin Patch (MR ASAP) was created and is currently on-going.[19] MR ASAP aims to assess functional outcomes at 90 days after administering transdermal GTN, within 3 h of symptom onset, to patients with ultra-acute ischemic stroke in the prehospital setting. 1,400 adult patients with suspected

stroke and systolic blood pressure ≥140 mmHg will be randomized to receive transdermal GTN (5 mg/day). Paramedics will administer the transdermal patch within 3 h of stroke onset in the prehospital setting. The MRS will be used to measure primary outcomes at 90 days. Summary of included clinical studies in ischemic stroke is presented in Table 1.

The Clinical Trials of Nitric Oxide in Myocardial Infarction

Myocardial infarction and treatment

Myocardial infarction (MI) occurs due to a prolonged stoppage of blood flow and oxygen to sections of the heart. Most often, this is due to a rupture with complete thrombosis of an unstable plaque consisting of white blood cells, cholesterol, and fat in the coronary arteries. Due to lack of oxygen, cardiac myocytes start to undergo necrosis resulting in injured heart muscle. To minimize damage from an MI, treatment and guidelines focus on achieving rapid reperfusion of the obstructed zone.[20] While early reperfusion is undoubtedly beneficial, IRI is a known complication of oxygen reperfusion that can result in free-radical mediated tissue damage to ischemic zones.[21] IRI damage to the microvasculature can continue to reduce blood flow to the ischemic tissue, leading to the "no-reflow phenomenon."[22] Many therapies have been introduced to combat IRI including but not limited to edaravone, vitamins, therapeutic hypothermia, and NO.[23] Studies show that NO and its derivatives such as inhaled NO, nitrites, and peroxynitrite modulate IRI of the myocardium.[24] For this review, we will examine the various NO derivatives and their efficacy in modulating MIs.

Inhaled nitric oxide

In 1999, the FDA approved inhaled nitric oxide (iNO) to treat persistent pulmonary hypertension in neonates. Since then, iNO has been widely adopted as a viable therapeutic to treat multiple disease processes.[25] In a few recent studies using feline models with intestinal ischemia-reperfusion, iNO administration resulted in decreased platelet activation and leukocyte adhesion causing increased coronary patency as well as improved blood flow after thrombolysis.[26,27] In a study led by Inglessis *et al*., they sought to assess if iNO could improve hemodynamic function in patients with right ventricular myocardial infarction (RVMI) and cardiogenic shock (CS). They enrolled 13 patients (7 males and 6 females) with acute inferior MI in this study and recorded hemodynamic measurements before, during, and after administration of iNO (80 ppm) in these patients. From their study, they concluded that iNO administration can leady to hemodynamic improvements in patients with RVMI and CS.[28] The trial NO for inhalation in ST-elevation MI (STEMI) tested if iNO reduces IRI in patients with STEMI. They recruited 250 STEMI patients to inhale oxygen with iNO or without iNO for 4 h following percutaneous revascularization. They concluded that iNO (80 ppm) administered for 4 h after STEMI revascularization was safe but did not reduce infarct size at 48–72 h.[29]

Nitric oxide donors

Since iNO had minimal effects in blunting IRI, researchers have turned toward exploring NO donor drugs as an alternative treatment option to iNO. Currently, two types of NO donor drugs are used clinically: sodium nitroprusside (SNP) and organic nitrates. These drugs can be administered in a variety of ways, some of which include slow-release oral forms, transdermal patches, and traditional intravenous (I.V.) forms. Many animal model studies have been performed to evaluate the efficacy of organic NO donor compounds on cardiac IRI injury. These animal studies show that NO donor compounds have variable effects on MI size and left ventricular function.[30] However, in human clinical trials with NO donors, the results are not as conclusive.[24]

Sodium nitrite

In the past, nitrite was considered a relatively inert breakdown product of NO, but evidence has emerged during the last decade, indicating that nitrite is a bioactive substance with promising therapeutic efficacy. Nitrites

Table 1: Summary of included clinical studies in ischemic stroke

NO donor (dose, route, duration)	Time from stroke onset to admission (h)	Outcome	Sample size (Tx: control)	Therapeutic effect of NO
Transdermal GTN (5 mg/24 h, 7 days)	≤48	Modified Rankin scale at 90 days	2000:2011	Did not improve functional outcome Bath *et al*. (2015)
Transdermal GTN (5 mg/24 h, 7 days)	≤6	Modified Rankin scale at 90 days	273	Improved functional outcome and fewer deaths Bath *et al*. (2015)
Transdermal GTN (5 mg/24 h, 7 days)	≤4	SBP at 2 h	25:16	Reduce SBP at 2 h Bath *et al*. (2013)
Transdermal GTN (5 mg/24 h, 4 days)	≤4	7-level modified Rankin scale at 90 days	568:581	Transdermal GTN does not improve functional outcome Bath *et al*. (2019)
Transdermal GTN (5 mg/24 h, 1 day)	≤3	Modified Rankin scale at 90 days	1400	The trial is ongoing van den Berg *et al*. (2019)

NO: Nitric oxide, GTN: Glyceryl trinitrate, SBP: Systolic blood pressure

are vasorelaxants with antiplatelet properties, both of which are enhanced by hypoxia.[31,32] The NIAMI trial investigated the effects of immediately administering sodium nitrite before reperfusion in patients with acute STEMI. 229 patients presenting with acute STEMI were randomized to receive either I.V. infusion of 70 mmol sodium nitrite (n = 118) or a matching placebo (n = 111) over 5 min immediately before primary percutaneous intervention (PPCI). The results showed that there is no reduction in infarct size with the I.V. administration of sodium nitrite administered immediately before reperfusion in patients with acute STEMI.[33] In another clinical trial, investigators used intracoronary injections in place of I.V. injection. Patients undergoing PPCI (n = 80) were randomized to receive intracoronary (10 ml) sodium nitrite (1.8 μmol) or NaCl (placebo) before balloon inflation. While the results showed a significant reduction in major adverse cardiac events as well as improved myocardial salvage index, there was no change in infarct size.[34]

Isosorbide dinitrate

In the 1990s, Hildebrandt *et al*. and Morris *et al*. investigated the therapeutic effect of adjunct therapy in treating MI. Hildebrandt *et al*.'s trial enrolled 100 consecutive patients with MI. They were admitted to the coronary care unit within 8 h after the onset of symptoms and given a streptokinase infusion of 1.5 million units for 1 h and a titrated dose of isosorbide dinitrate (ISDN) or placebo for 48 h. On the other hand, Morris *et al*.'s trial aimed to assess the possible benefits of I.V. ISDN in acute myocardial infarction (AMI) and oral isosorbide mononitrate in sub-AMI. The trial enrolled 316 patients presenting with AMI. Both of the clinical trials concluded that there was no reduction in infarct size. However, Morris *et al*. also found evidence of a heterogeneous effect in different subgroups of acute infarction as well as in different groups of patients.[35,36]

Sodium nitroprusside

SNP is a well-known venue and vasodilator used in clinical practice to lower BP. In the body, it functions as a prodrug, reacting with sulfhydryl groups on erythrocytes, albumin, and other proteins to release NO.[37] NO results in reduced vascular tone in the muscular arteries at the tissue level.[38] In a preliminary study with 125 patients, 101 males and 24 females, suffering from AMI, measured plasma NO levels were found to be severely reduced (0 nmol/ml; median). Since the results from this study showed severely reduced NO levels, Ghosh *et al*. theorized that increasing systemic plasma NO levels by using SNP "pad" could prevent AMIs. They created a study to test this hypothesize and enrolled 8,283 volunteer participants with different types of cancers. Cancer patients were chosen due to their higher risk of death from AMI. The authors concluded that the use of SNP "pads" normalized (<4.0 mM) the plasma NO levels and resulted in a significantly reduced death rate due to AMI among the participants compared to the death rate due to AMI in the normal population.[39] Summary of included clinical studies in MI is given in Table 2.

Summary and Future Direction

Both clinical and animal studies have demonstrated a positive effect of NO on the treatment of IRI when

Table 2: Summary of included clinical studies in myocardial infarction

NO donor (dose, route, duration)	Time from MI onset to admission (h)	Outcome determination	Sample size (Tx: control)	Effect of NO donor on outcome versus control
Inhaled NO 80 ppm for 10 min	49±11	Hemodynamic measurements	13	NO inhalation results in acute hemodynamic improvement Inglessis *et al*. (2004)
Inhaled NO 80 ppm for 4 h	≤6	LV size (IS/LV mass)	122:127	Did not reduce infarct size relative to absolute LV mass at 48-72 h Janssens *et al*. (2018)
Sodium nitrite 70 umol infusion for 5 min	≤12	CMR % LV mass 6-8 days postinfarct	118:111	No reduction in infarct size Siddiqi *et al*. (2014)
Sodium nitrite 1.8 μmol intracoronary injection	≤12	Creatine kinase release, troponin T release, and cardiac MRI on day 2	38:38	No reduction in infarct size Daniel *et al*. (2014)
Isosorbide dinitrate 1.0-10.0 mg/ mL infusion for 48 h	≤8	CK-MB every 4 h for72 h	50:49	No reduction in infarct size when reperfusion confirmed Hildebrandt *et al*. (1992)
Isosorbide dinitrate 1.0-6.0 mg/h infusion for 24 h	≤24	αHBDH blood samples every 12 h on days 1 and 2 and daily on days 3, 4, and 5	150:151	No reduction in infarct size Morris *et al*. (1995)
SNP 0.28 mmol 3 years	-	Death rate	4846:3437	SNP "pad" significantly reduced the death due to AMI Ghosh *et al*. (2014)

LV: Left ventricle, NO: Nitric oxide, MRI: Magnetic resonance imaging, AMI: Acute myocardial infarction, SNP: Sodium Nitroprusside, IS: Ischemic stroke, CK-MB: Creatine kinase-MB, CMR: Cardiac magnetic resonance imaging

using different NO donors (iNO, transdermal GTN, I.V. sodium nitrite). However, there does not seem to be a consistent improvement in functional outcomes in patients with presumed stroke and MI. The differences between the studies may be due to better study design and eliminating confounding variables. Future clinical trials can focus on using iNO in the treatment of ischemic stroke as no such study exists. Other future studies can focus on using different concentrations of NO, determining administration time interval for NO, and identifying specific population groups that may benefit from NO therapy.

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Conflicts of interest

There are no conflicts of interest.

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