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Nitrates and Nitrites in the Treatment of Ischemic Cardiac Disease

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Abstract

The organic nitrite, amyl of nitrite, was initially used as a therapeutic agent in the treatment of angina pectoris in 1867, but was replaced over a decade later by the organic nitrate, nitroglycerin (NTG), due to the ease of administration and longer duration of action. The administration of organic nitrate esters, such as NTG, continues to be used in the treatment of angina pectoris and heart failure during the birth of modern pharmacology. The clinical effectiveness is due to vasodilator activity in large veins and arteries through an as yet unidentified method of delivering nitric oxide (NO), or a NO-like compound to vascular smooth muscle cells. The major drawback with NTG administration is the rapid development of tolerance; and with amyl of nitrite, the duration and route of administration. Although amyl of nitrite are no longer used in the treatments of hypertension or ischemic heart disease, the nitrite anion has recently been discovered to possess novel pharmacologic actions such as modulating hypoxic vasodilation and providing cytoprotection in ischemia-reperfusion injury. Although the actions of these two similar chemical classes (nitrites and organic nitrates) have often been considered to be alike, we still do not understand their mechanism of action. However, the recent discovery that the nitrite anion, derived from either sodium nitrite or an intermediate NTG form, may act as a storage form for NO and provides support for investigating the use of these agents in the treatment of ischemic cardiovascular states. We review what is presently known about the use of nitrites and nitrates, the potential uses of these agents, and their mechanisms of action.

Keywords

Nitrites; Nitrates; Nitroglycerin; Nitric oxide; Ischemia-Reperfusion Injury; Hypoxia; Xanthine oxidoreductase; Mitochondrial aldehyde dehydrogenase

Cardiovascular diseases (CVD) are a group of disorders including congestive heart failure (CHF) and ischemic heart disease that are becoming the leading cause of morbidity and mortality in the world [1,2]. Initial treatment of both disorders began during the latter 1800s with organic nitrates or with organic nitrites following animal and human experiments of Brunton and Murrell [3,4]. Ischemic myocardium results when the supply of oxygenated blood through the coronary arteries becomes inadequate to meet the demands of cardiac muscle, leading to myocardial anoxia [5]. CVD can result from a quantitative or functional nitric oxide (NO) deficiency that can limit NO-dependent signal transduction pathways to the detriment of normal cellular function, and as such, delivery of low concentrations of exogenous NO would be an attractive therapeutic option [6]. Current indications for nitrate therapy include chronic, stable angina pectoris, unstable angina pectoris, complications of acute myocardial infarction,

and ventricular unloading in acute and chronic CHF [7]. Nitrates are also used in the perioperative period by anesthesiologists and intensivists to control systemic and pulmonary arterial pressure [7–10]. Due to similar historical introductions of the organic nitrate ester, nitroglycerin (NTG) and the organic nitrite, nitrite of amyl, both with comparable therapeutic actions, we review these therapeutic introductions and the novel mechanisms of action.

History

Sir Thomas Lauder Brunton first used amyl nitrite in the treatment of angina pectoris in 1867 [3]. As a medical student, Brunton had become aware of prior clinical findings of Benjamin Ward Richardson that inhaled amyl nitrite rapidly increased the action of the heart [11], and also the unpublished observations of Arthur Gamgee demonstrating that amyl nitrite greatly lessened ‘arterial tension’ in both animals and man [3]. During the same period in which Brunton used amyl nitrite, another British physician, William Murrell, began using the organic nitrate, GTN, in the treatment of angina pectoris [4]. With NTG therapy, patients would obtain relief from angina with some patients also reporting that their angina could be aborted by taking the drug at the onset of symptoms [4]. Murrell also worked with Fancourt Barnes and compared the effects of amyl nitrite with NTG, where it was observed that the actions of these drugs differed in the time of onset and duration. It was concluded that NTG would be more clinically useful than amyl nitrite [12]. Murrell’s use of NTG, the world’s first synthesized drug, in the treatment of angina pectoris is still in use 140 years later [12,13].

In the 1920s, Richard Bodo used the Starling’s heart–lung preparation to determine the effects of two nitrite preparations on coronary flow [14] as earlier work in myocardial studies demonstrated that nitrite preparations produced vasorelaxation [15–17]. Titrated doses of sodium nitrite would produce dose-dependent increases in total coronary flow without a return to control levels between doses [14]. In contrast, amyl nitrite would produce greater responses in coronary flow, but with complete recovery between doses [14], findings that confirmed the earlier observations of Cow [17]. These findings suggested that although amyl nitrite could produce a greater response in flow, a more prolonged effect could be obtained with the inorganic nitrite, sodium nitrite.

The use of nitrates, especially NTG, suggested that coronary vasodilation [18–20] might be the mechanism to improve the symptoms and electrocardiographic (EKG) changes of angina pectoris [21,22]. However, contemporary studies reported a high incidence of ‘placebo effect’ when assessing symptom relief [23], or that NTG -induced vasodilation could not be demonstrated [24,25], nor significant changes in measured coronary sinus oxygen content [24,26,27].

By the 1930s, nitrites and nitrate esters were established treatments for angina and hypertension [28–31]. Weiss and Ellis performed detailed clinical studies of the cardiovascular effects of sodium nitrite in 1933 in normal individuals, in patients with hypertension, and in patients with renal disorders [29]. In these studies, the degree of systolic pressure decrease following administration of sodium nitrite was dependent upon the level of arterial tone, but administration of sodium nitrite did not improve urinary output or creatinine clearance in patients with renal disorders. Moreover, Weiss suggested that the use of sodium nitrite in the treatment of hypertension would not be efficacious and may be dangerous [29]. Although nitrites generally were beneficial in patients with angina, in some patients the use of nitrites was not beneficial and caused untoward effects [3,23,32]. Contro and colleagues observed a paradoxical action following inhalation of amyl nitrite in patients with coronary artery disease, in that EKG changes resembling myocardial ischemia would occur after administration of amyl nitrite due to a sudden drop in blood pressure followed by coronary insufficiency [5]. Finally, the authors suggested that the nitrite could be used as a diagnostic test in patients with borderline

or questionable history of coronary artery disease [5]. Interestingly, NTG was also reported to induce EKG changes following administration of this organic nitrate [33]. However, subsequent studies by Kerber and Harrison were unable to reproduce the reported EKG changes with NTG, but were able to confirm with coronary angiography that EKG changes with amyl nitrite were due to myocardial ischemia [34]. Amyl nitrite and NTG have been evaluated in patients with essential hypertension [35]. NTG was able to reduce systolic and mean arterial pressures and cardiac output, but had little effect on diastolic pressure and total peripheral resistance [35]. Amyl nitrite decreased systolic, diastolic, and mean arterial pressures and peripheral vascular resistance, and increased heart rate and cardiac output [35]. By the 1970s, nitrite preparations were infrequently used in the medical management of angina pectoris or hypertension [36]. Moreover, amyl nitrite has been found to have abuse potential resulting in significant methemoglobinemia, blindness, and death [37–40]. Although nitrite is no longer used for the treatment of ischemic heart disease or systemic hypertension [41], it still has benefit in the treatment of cyanide toxicity [42]. All clinically active nitrites and nitrates are esters of nitric or nitrous acid with the exception of sodium nitrite. The nitrites/nitrates are classified as agents that directly relax vascular smooth muscle, but can also relax other smooth muscles such as bronchial, ureteral, and uterine smooth muscle [43–46]. These similar historical descriptions of NTG, amyl nitrite, and sodium nitrite, in their ability to produce direct vasorelaxation, were subsequently found to induce direct vasorelaxation via vascular NO formation [47].

Vascular NO formation

NO formation by endothelial NO synthase (NOS) plays an important role in the regulation of vasomotor tone in the pulmonary and systemic vascular beds (Fig. 1) [48–51]. The importance of NO in the regulation of vasomotor tone has been demonstrated in experimental animals and in human subjects by the use of NOS inhibitors [48,50–52]. NO is also produced by NOS-independent mechanisms [53–55]. Nitrite can be produced by oxidation of NO under physiologic conditions (Fig. 1) [56–58]. However, NO generation by NOS may be rapidly depleted in ischemic conditions, since NOS is dependent upon the availability of oxygen [59, 60]. Once released from the endothelium, NO diffuses into vascular smooth muscle cells inducing vasodilatation and into the blood stream where it inhibits platelet aggregation and inflammation (Fig. 1) [61–63]. NO is an important paracrine molecule for the maintenance of a healthy endothelium [64,65]. Loss of endogenous NO activity has a number of detrimental actions, most notably, vasoconstriction, increased activity and adherence of platelets, and accumulation of inflammatory cells at sites of endothelial damage. When the endothelium deteriorates or becomes dysfunctional, blood flow is disturbed and vessels become obstructed, leading to ischemia of downstream organs including the heart [66–71]. Endothelial damage is associated with most forms of CVD, including hypertension, coronary artery disease, CHF, peripheral artery disease, diabetes, chronic renal failure, and pulmonary hypertension [72–76]. Endothelial function is altered in the presence of cardiovascular risk factors, suggesting that endothelial dysfunction is likely to be an important first step in CVD [77–86]. Endothelial dysfunction arises from down-regulation of endothelial NOS expression and activity, and uncoupling of NOS generating free radicals [87]. The delivery of exogenous NO initially would be an attractive therapeutic option if this intervention could slow down the progression of endothelial dysfunction and reduce the risk of CD [6,87,88], as the administration of organic nitrates and nitrites have been shown to have beneficial effects in CVD [3,4,89–94]. The pharmacologic actions of these two classes (nitrites and organic nitrates) have been considered to be similar [95], however recent interest in the chemical classes of agents that liberate NO have been shown to have important differences [96,97]. Historical work by Krantz and colleagues demonstrated that the rapid-acting nitrites and organic nitrates, amyl nitrite, NTG, and isobutylglycollate nitrate, could inhibit the ATPase activity in the rabbit aorta, whereas sodium nitrite had little or no effect [98]. It was reported that oxygen uptake was inhibited by

sodium nitrite and NTG, whereas amyl nitrite, erythritol tetranitrate, and octyl nitrite did not significantly interfere with oxygen uptake in arterial tissue when these agents were administered in therapeutic levels [99]. These early studies suggested that these two classes of nitrovasodilators have different mechanisms of action. Additional studies have demonstrated different enzymatic requirements for NO generation by organic nitrates when compared to nitrites [100]. Moreover, studies have shown that a reduced endothelial NO production following administration of organic nitrates, such as NTG, may be a mechanism of nitrate tolerance [101–103], but that amyl nitrite has different vascular actions and hemodynamic effects and produces less tolerance when compared to NTG [97]. These findings were subsequently confirmed in other studies [34], and although NTG was reported to exacerbate EKG changes during exercise [33], Kerber and Harrison were unable to reproduce this effect with NTG [34]. These findings suggest that the different results observed with NTG and amyl nitrite are probably due to the findings that NTG has a direct action on veins to reduce preload, whereas amyl nitrite reduces after load that decreases coronary flow [34]. Although the literature during this time suggests that the cardio-circulatory actions of the nitrites and organic nitrates were similar, important differences in the route of administration could have influenced these circulatory actions. Rapid introduction of amyl nitrite by inhalation or of NTG by intravenous administration could result in profound arteriolar dilation and a marked fall in systemic arterial pressure. The dilation of systemic arterial and venous beds leading to baroreceptor stimulation results in marked reflex increases in heart rate [104,105]. However, slower introduction of these agents using sublingual NTG or oral sodium nitrite administration would result in smaller decreases in arterial pressure and reductions in central venous pressure producing peripheral venous pooling, a reduction in stroke volume and in cardiac output [105–107]. Although the fundamental action of these two classes is generalized as direct vasodilation, relief of angina pectoris with sublingual NTG appears to be more related to direct venodilation, whereas inhalation of amyl nitrite abates angina from direct and marked arteriolar dilation, and that reduction of myocardial oxygen consumption, rather than augmentation of diminished coronary blood flow, is the common mechanism by which anti-anginal measures can relieve ischemic pain [108]. To further confirm the beneficial venodilatory effects of NTG, Ganz and Marcus used pacing-induced tachycardia to induce angina and observed that only administration of intravenous, but not intracoronary, NTG was effective in relieving ischemia, suggesting that the direct action of NTG on the coronary vascular bed has little role in the anti-anginal effect of the drug but requires an action on the systemic circulation [109]. Although nitrite is no longer used in the treatment of ischemic heart disease or in systemic hypertension [36,41], it still has benefit in the treatment of cyanide toxicity [42], along with recently discovered actions in mediating or modulating hypoxic vasodilation [54,110,111], and the ability to induce cytoprotection in ischemia-reperfusion injury in a number of organ systems [112–114].

Mechanisms of Action for the Nitrates and Nitrites

NTG and amyl nitrite have been used in the treatment of angina pectoris and HF for 140 years [3,4], and it is generally believed that the therapeutic effect of these drugs involves the release of NO from nitrite (Fig. 1), the activation of guanylyl cyclase, and relaxation of blood vessels [4,87,115–117]. Studies have shown that bioactivation of NTG requires thiols or sulfhydryl-containing compounds and that NO or NO-like compounds are believed to be the biologically active species [47,118–120,164]. Interactions with NTG and sulfhydryl-containing receptors are necessary for vascular smooth muscle relaxation and repeated administration of NTG produces sulfhydryl depletion and the development of tolerance [101,120,121]. Subsequent studies have demonstrated the release of NO following the decomposition of an intermediate S-nitrosothiol [47]. Additional studies suggest that an enzymatic mechanism may be responsible for the bioactivation of NTG. However, these enzymatic systems could not catalyze the selective formation of 1,2-glyceryl dinitrate and nitrite from NTG, and moreover, no

association was observed between the development of enzymatic tolerance and tolerance to NTG [122–128]. However, the discovery that mitochondrial aldehyde dehydrogenase (mtALDH) generates 1,2-glyceryl dinitrate and nitrite from NTG, that this reaction requires a reducing thiol cofactor, and that the activity of this enzyme is reduced in NTG tolerance, suggest that this pathway is responsible for NTG bioactivation in vascular smooth muscle [129,130]. However, one difficulty with these studies has been an inability to detect NO as a byproduct of NTG metabolism [131]. Moreover, the generation of NO was observed when the concentrations of NTG exceeded therapeutic values [103,132–136]. A potential solution has been proposed that once NTG is bioactivated within the mitochondria, nitrite or an additional action of mtALDH generates the vasodilatory NO bioactivity [13]. One suggested mechanism for this vasodilatory activity is that S-nitrosoglutathione is formed by the reaction of reduced glutathione and nitrite [137,138]. This molecule subsequently undergoes biotransformation to S-nitrosocysteine [130,139] that can release NO [140]. However, excessive amounts of NTG or S-nitrosothiols can dysregulate protein S-nitrosylation and contribute to cellular dysfunction and disease [141]. Chronic NTG administration has been shown to result in acetylcholine-induced coronary vasoconstriction rather than relaxation [142,143] and induce endothelial dysfunction [144]. An early event in the pathophysiology of atherosclerosis are the impairment of endothelial function or endothelial dysfunction that develops before structural changes of intimal hyperplasia or lipid deposition occur [145]. Moreover, reduced bioavailability of NO occurs through reduced endothelial NOS expression and activity [145], which can be evidenced by abnormal vascular responses to an acetylcholine challenge [146,147]. Therefore, NO deficiency is linked to CVD processes and provides justification for the use of effective NO replacement therapy.

Nitrates

The most commonly used agents at this time include isosorbide dinitrate, isosorbide-5-mononitrate, and NTG which are effective in reducing ventricular preload by increasing peripheral venous capacitance [148–151]. These drugs can also decrease pulmonary and systemic vascular resistances, but require higher doses than those needed for the increase in venous capacitance [152–157]. These agents can reduce ventricular filling pressure, wall stress and myocardial oxygen consumption [158], and may also improve systolic and diastolic ventricular function by improving coronary flow in patients with ischemic cardiomyopathy. However, there is, as yet, no convincing evidence that organic nitrates improve mortality in patients with acute myocardial infarction [159,160]. The limitations of this class of agents are well known and potentially include adverse hemodynamic effects, drug tolerance, lack of selectivity, and limited bioavailability [87].

Studies in the literature provide evidence that vasorelaxant responses to NTG are mediated by the formation of NO or a closely related molecule [47,161–163]. However, the mechanism of this vasorelaxant response to NTG is uncertain. Although studies in the literature indicate that NO contributes to the activation of guanylate cyclase and vascular smooth muscle relaxation [164,165], other studies suggest that vasorelaxant responses to NTG may be independent of NO release and cyclic guanosine monophosphate (cGMP) formation [129].

Role of Mitochondrial Aldehyde Dehydrogenase

It has been reported that ALDH2 catalyzes the formation of glyceryl dinitrate and nitrite from NTG, leading to the production of cGMP and vasorelaxation [129,164,166–171]. Moreover, it has been suggested that the nitrite formed from NTG metabolism may be further metabolized to NO and/or converted to a S-nitrosothiol [172]. Although it has been reported that ALDH2 plays an important role in the bioactivation of NTG, the role of this enzyme in the reduction of nitrite to vasoactive NO has only recently been determined. We have shown in rats treated with L-NAME to block NOS, that decreases in systemic arterial pressure in response to

intravenous injections of sodium nitrite were attenuated by cyanamide, an inhibitor of ALDH2 [173]. Moreover, these data were consistent with the results of Ohtake and colleagues [172]. The results of these studies provide support for the hypothesis that ALDH2 plays an important role in the bioactivation of NTG [129,166–171]. In summary, ALDH2 plays an important role in the bioactivation of NTG and nitrite.

Nitrites

Recent studies provide support for the concept that the nitrite anion represents a storage form of NO that can have therapeutic effects [174–176]. Although nitrite was believed to be an inactive end-product of NO metabolism that reflects endothelial function, the vasodilator actions of nitrite were recognized as early as 1867 by Brunton who used amyl nitrite to treat angina [3]. Cardiovascular responses to sodium nitrite have been investigated in both the last century and in this century [29,106,110,168,176,177]. The ability of sodium nitrite to relax isolated arteries was first reported in 1953 and the effects of nitrite on guanylyl cyclase activity and cGMP levels were documented by Ignarro et al and others [47,118,164,178–181]. The mechanism by which nitrite is reduced to NO is uncertain but the reduction of inorganic nitrite to NO can be mediated by enzymatic mechanisms and by nonenzymatic disproportionation at low pH (Fig. 1) [110,132,173]. Moreover, it has also been reported that vasodilator responses to sodium nitrite can be attenuated by allopurinol, suggesting that xanthine oxidoreductase (XOR) can play a role in bioactivation of nitrite (Fig. 1) [117,172,173].

Role of Xanthine Oxidoreductase

The mechanism by which NTG activates guanylyl cyclase is uncertain [167–169]. The nitrite that is formed from the reaction of NTG with ALDH2 can be reduced to vasoactive NO or a NO-like compound that activates guanylyl cyclase [167,168]. XOR is a ubiquitous enzyme that can reduce nitrite to NO, and under severe conditions in the rat heart, large amounts of NO can be generated (Fig. 1) [53,182,183]. XOR is widely distributed in mammalian tissues and is a key enzyme in purine metabolism. Although XOR can reduce nitrite to NO, and that nitrite reduction is greatly enhanced under hypoxic and ischemic conditions in the rat [184], the effects of XOR inhibitors on vasodilator responses to sodium nitrite are uncertain, and studies in the literature show no inhibitory effect [167,168]. XOR has been shown to reduce nitrite to NO and it has been reported that allopurinol inhibits responses to sodium nitrite in the rat (Fig. 1) [54,185–187]. We have shown that systemic vascular responses to the nitrite anion were inhibited by allopurinol in a dose that did not alter responses to sodium nitroprusside and these findings support the hypothesis that XOR contributes to the activation of nitrite in the rat and that the nitrite anion represents a storage form of NO (Fig. 1) [184]. Moreover, in a recent study presented at the Second International Meeting on the Role of Nitrite in Physiology, Pathophysiology and Therapeutics, National Institutes of Health, Bethesda, Maryland, allopurinol was shown to attenuate decreases in systemic arterial pressure in response to systemic administration of sodium nitrite in L-NAME-treated rats [172] and are consistent with the hypothesis that XOR plays an important role in mediating vasodilator responses to sodium nitrite [47,119,188]. Furthermore, the inhibitory effects of cyanamide and of allopurinol on responses to sodium nitrite were shown to not be additive, suggesting that XOR and ALDH2 act in a parallel manner to reduce nitrite to vasoactive NO in the systemic vascular bed of the rat. These data are in agreement with previous studies and provide support for the hypothesis that ALDH2 plays a role in the bioactivation of nitrite (Fig. 1) [167–169].

Role of Xanthine Oxidoreductase and Mitochondrial Aldehyde Dehydrogenase

The hypothesis that XOR contributes to the bioactivation of NTG by reducing nitrite derived from the activity of ALDH2 was recently examined in studies with allopurinol and cyanamide. We observed that responses to NTG were not attenuated by allopurinol in doses that

significantly decrease responses to sodium nitrite [173]. The observation that responses to NTG are not altered at a time when responses to sodium nitrite were attenuated suggests that the reduction of the nitrite anion to vasoactive NO did not contribute to the vasodilator responses to NTG in the pulmonary and systemic vascular beds of the intact chest rat. Moreover, when comparison of vasodilator responses to NTG and sodium nitrite was plotted, NTG was ~1000 fold more potent than sodium nitrite in decreasing pulmonary and systemic arterial pressures in the rat [173]. These findings suggested that the conversion of nitrite to vasoactive NO, even if all three-nitrate groups of NTG were reduced to NO, would not be able to account for the vasodilator activity of NTG in the intact chest rat [173]. These findings are consistent with the results of studies showing that vasorelaxant responses to NTG can occur independently of its NO-releasing properties in isolated vessels [189]. These observations are consistent with this hypothesis, and that the identity of the chemical species formed from NTG that activates guanylyl cyclase in the intact vascular bed continue to be uncertain [47,131,164].

Conclusion

In this review we discussed the history and actions of organic nitrates and organic/inorganic nitrites in clinical medicine for the treatment of ischemic heart disease. Although the nitrovasodilators can substitute for endogenous NO, the pathway of bioactivation substantially differs with nitrites and organic nitrates. In the case of organic nitrates such as NTG, NO is only formed if thiols are present as a cofactor. Nitrites such as amyl nitrite and sodium nitrite require reduction of nitrite anion to vasoactive NO and that this bioactivation can be mediated by XOR or ALDH2. The observation that responses to NTG were not altered at a time when responses to sodium nitrite were attenuated, suggests that the reduction of the nitrite anion formed from NTG to NO does not contribute to vasodilator responses to NTG in the pulmonary and systemic vascular beds of the intact chest rat. Finally, recent findings suggest that both XOR and ALDH2 can act in a parallel manner to reduce nitrite to vasoactive NO [172,173]. Nitrates are still the most widely used drugs for the treatment of ischemic heart disease and recent evidence now suggests that nitrites have a beneficial effect in protecting the heart and other organs from ischemia.

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Nitrite (NO_2^-) Reduction to Vasoactive Nitric Oxide

Figure 1.

Endothelial nitric oxide synthase (eNOS) generates nitric oxide ($\text{NO}^\bullet$) and L-citrulline from L-arginine. Circulating $\text{NO}^\bullet$ in the presence of oxygen (O_2) can be oxidized to form nitrite (NO_2^-). Circulating NO_2^- can be reduced by deoxyhemoglobin to $\text{NO}^\bullet$ and methemoglobin under ischemic conditions. Modified from Lefer, D. (2006). Nitrite therapy for protection against ischemia-reperfusion injury.