

Review

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Review

Review of the Role of Mitochondria in the Actions of Nitroglycerin and Redox Disturbances Underlying Diseases it Treats

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Abstract: The purpose of this paper is to review the involvement of mitochondria in the actions of nitroglycerin (glycerol trinitrate -GTN) and the redox disturbances underlying diseases it treats. The paper begins by explaining complex I as the initial site of formation of superoxide and the process whereby it leads to the development of glutathione dependent tolerance. Next, the paper describes new formulations for preventing tolerance. The paper ends by illustrating diseases that may benefit from treatment with novel glutathione based GTN formulations and treatment approaches.

Keywords: nitroglycerin; glutathione; mitochondria; complex I; eNOS; nitric oxide; S-nitrosylation; S-glutathiolation; superoxide; acute heart failure; right heart failure; diuretic resistance; refractory angina; NLRP3 inflammasome

Introduction

GTN was first discovered in 1847 by Sobrero [1]. Tolerance seen in its treatment of patients was first described in 1888 [2]. Over the ensuing years many hypotheses to explain and eliminate tolerance in the treatment of cardiovascular diseases have been advanced. Recently superoxide from mitochondria in response to GTN treatment has been discovered [3]. The purpose of this paper is to review the involvement of mitochondrial complex I as the site of synthesis of the first superoxide radical in response to GTN and the mechanism whereby it leads to glutathione (GSH) dependent tolerance. The paper also will explain the rationale for GSH based GTN formulations and approaches, that not only address tolerance, and vasodilator heart failure treatment failures involving redox induced diuretic resistance, but also address the rationale for their use to target underlying redox disturbances of other diseases treated by GTN.

Nitric Oxide Is the Cause of GSH Dependent GTN Tolerance

The cause of GTN tolerance is nitric oxide (NO). GTN tolerance is a process that begins at mitochondrial complex I with its uncoupling by S-nitrosylation [4,5] from NO donated and generated [6–8] by GTN. The resulting superoxide formed depletes GSH by S-glutathiolation thereby leading to further superoxide which is GSH suppressible [4,9,10]. This process ends at NADPH oxidase. In between mitochondrial complex I and NADPH oxidase there is uncoupling of aldehyde dehydrogenase II (ALDH II) and eNOS, that involves its S-glutathiolation [13], which follows complex I uncoupling and substrate depletion by arginase II [6,12], all of which is mediated by ROS and RNS from uncoupled complex I. This is followed by activation of protein PKC [6,13] which completes the process by activating NADPH oxidase with superoxide production from a third and final site.

Therefore, the site of formation of the first superoxide radical is mitochondrial complex I and NADPH oxidase is the site of the last superoxide radical's formation. The net effect of superoxide from all 4 sites is formation of peroxynitrite [6,14] in place of NO from ALDH-2 and EDNO from

eNOS. Tolerance with loss of vasodilator function is the result. This entire GSH dependent-reversible process is illustrated and summarized in Figure 1 and discussed in complete detail in reference [6].

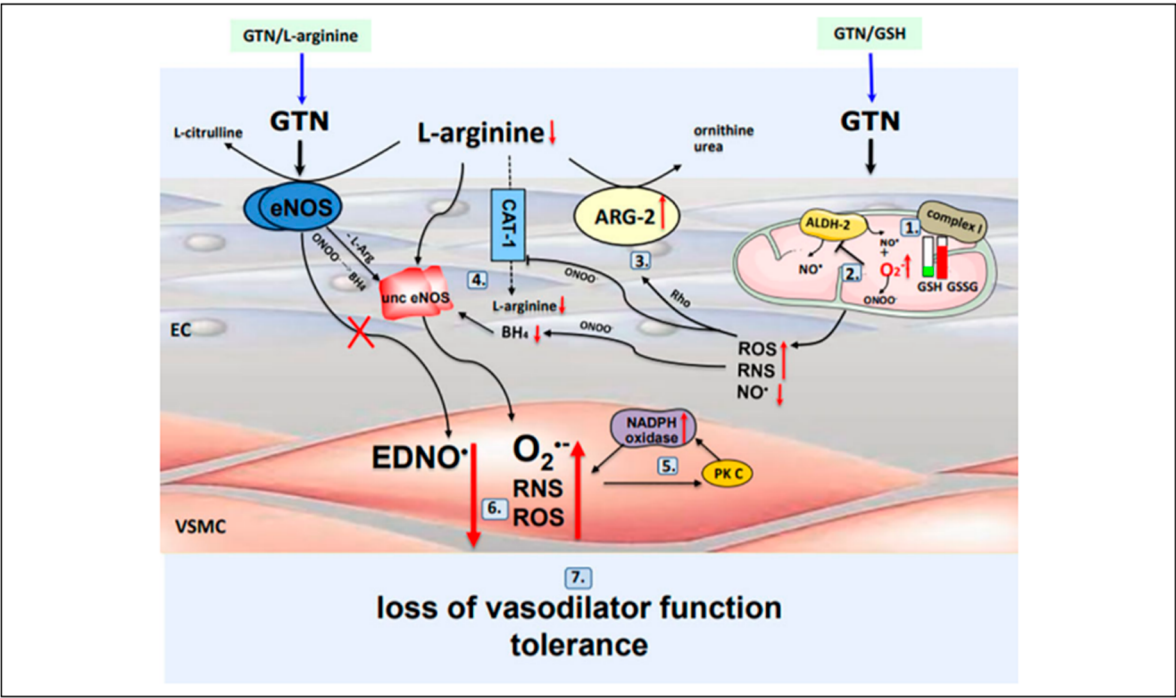


Figure 1. This figure illustrates a 7 component process that (1) begins at complex I, NO from GTN either from denitration or eNOS or both and leads to inhibition of complex I as a result of calcium dependent depletion of GSH and ROS and RNS generation. (2) ALDH-2 mediated bioconversion of GTN to NO is inactivated by ROS and RNS from step1, thereby leading to a loss of NO from bioconversion. (3) RNS from ALDH-2 upregulate arginase II via activation of RhoA/Rho kinase and reduce L-arginine availability to eNOS. (4) eNOS uncoupling from both, L-arginine and BH4 depletion further promotes superoxide production. (5) Superoxide from eNOS activates PK C and PK C subsequently activates NADPH oxidase, a fourth site of superoxide. (6) Superoxide from all 4 sites induces formation of peroxynitrite and related radicals in place of NO from ALDH-2 and EDNO from eNOS. (7) Tolerance is the end result of loss of vasodilator function. EC indicates endothelial cells; ARG-2, arginase-2; BH4, tetrahydrobiopterin; unc eNOS, uncoupled eNOS; GSH, glutathione; GSSG, glutathione disulfide; EDNO, endothelium-derived NO; PK C, protein kinase C; ALDH-2, aldehyde dehydrogenase-2; VSMC, vascular smooth muscle cells; ROS, reactive oxygen species; RNS, reactive nitrogen species; CAT-1, cationic amino acid transporter 1. 1.-7. Summary items (see in text).

A Chronology of Studies Showing the Actions of GTN and Tolerance

Are GSH Dependent

Beginning as early as 1969, Needleman showed the vasodilator actions of nitroglycerin (GTN) are glutathione (GSH) dependent [15]. This was followed by studies showing GTN tolerance involves depletion of sulphydryl groups and lead to the “sulphydryl-depletion hypothesis” of GTN tolerance [16]. In 1983 it was shown that the hemodynamic actions of GTN could be potentiated by n-acetylcysteine (NAC), a GSH prodrug, in patients undergoing cardiac catheterization for evaluation of chest pain [17]. In 1987 it was shown that NAC prevents GTN tolerance in heart failure [18]. In 1995, the parenteral administration of 4.2 grams of GSH over a 7 day period was found to normalize primary tolerance to the rheologic effects of GTN in 40 patients with diabetes [19]. A 1998 study of GSH administered directly into the angiographically normal coronary arteries of 26 patients was found to enhance the epicardial coronary vasodilator response to GTN in addition to suppressing the coronary constrictor response and enhancing the increase in coronary flow response to acetylcholine

[20]. A 1999 study of patients with femoral artery atherosclerosis revealed prevention of GTN induced vasoconstrictor responses, similar to those seen in diabetics in 1995 [19], with intra-arterial GSH pretreatment [19]. Finally, in 2006 it was shown tolerance to GTN involves respiratory complex I and can be prevented by GSH [5].

GSH Based GTN Formulations and Treatment Approaches that Address Tolerance and the Redox Disturbances Underlying Diseases Treated by GTN

Based on the foregoing discussion of studies supporting the mechanism of tolerance illustrated in Figure 1, one formulation and two approaches to administering GTN in combination with GSH are proposed for intravenous, sublingual and transdermal dosing of GTN in the treatment of cardiovascular diseases.

Table 1 illustrates a new formulation of GTN for intravenous dosing that replaces 5% dextrose with 5% GSH. For maintaining pH in the range of 3-5, 2 % L-arginine is used as a buffer. This has been found to maintain a pH of 4 for a year or more. In addition to functioning as a buffer, L-arginine is anticipated to prevent eNOS uncoupling from system y+ transporter oxidative dysfunction leading to L-arginine depletion at the site of eNOS activity as a result of supplying L-arginine via passive diffusion as previously described [6,22,23]. Therefore, L-arginine performs a dual-use function. This new IV formulation, similarly dosed as the current dextrose based GTN formulation, would replace that dextrose formulation in the treatment of acute decompensated heart failure and controlling right heart failure which complicates the use of left ventricular assist devices in the treatment of advanced heart failure as previously discussed [6]. Finally it should be noted that this new formulation’s calculated osmolality is 278 mOsm/L. After adjusting for the small amount of excipients from GTN, this new formulation is is osmolar with serum which makes it suitable for long term continuous dosing.

Table 1 Current reformulation of nitroglycerin in dextrose 5% (A) and proposed reformulation of nitroglycerin in GSH (B)

(A) Nitroglycerin in dextrose 5%				
	Composition		Osmolarity (mOsmol/L) (calc)	pH
	Nitroglycerin (µg/mL)	Dextrose hydrous, USP (g/L)		
25 mg nitroglycerin in 5% dextrose injection	100	50	428	4.0 (3.0–5.0)
50 mg nitroglycerin in 5% dextrose injection	200	50	440	4.0 (3.0–5.0)
100 mg nitroglycerin in 5% dextrose injection	400	50	465	4.0 (3.0–5.0)
(B) Proposed reformulation of nitroglycerin in GSH 5%				
	Nitroglycerin (µg/mL)	GSH ^a , USP (g/L)	Osmolarity (mOsmol/L) (calc)	pH
25 mg nitroglycerin in 5% GSH injection	100	50	428	4.0 (3.0–5.0)
50 mg nitroglycerin in 5% GSH injection	200	50	440	4.0 (3.0–5.0)
100 mg nitroglycerin in 5% GSH injection	400	50	465	4.0 (3.0–5.0)

GSH glutathione, GTN glyceryl trinitrate, USP United States Pharmacopeia

^aBuffered to pH 3–5 with L-arginine. This reformulation involves dissolving 50 g GSH (USP grade) in 800 mL of water. Once this dissolves, 20 g of L-arginine are added and dissolved. Next GTN concentrate, 5 mg/mL, is added (20, 40, or 80 mL) as indicated in Table 1B. The total volume is increased to 1 L. The pH is checked to be sure it is in the range of 3–5. If not, it can be further adjusted by adding NaOH or HCl. Once this is complete, the solution is passed through a 22-µm Millipore filter to insure it is free of impurities and bacteria. Then it is transferred to airtight sealed glass bottles, generally 250 mL. This is referred to as “sterile fill,” and it is a process that can be done by compounding pharmacies. It is also suitable for commercial product manufacture (Alcami, personal communication, 2020). At the pH of 3–5, GTN is stable indefinitely. At pHs below 3, GTN undergoes slow degradation by one chemical reaction, and at pH greater than 5, it slowly degrades by a different reaction

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For sublingual GTN dosing, a recent study has shown showing GSH can be administered sublingually using slow melt sublingual tablets [24]. Based on previous use of buccal administration of GTN [25], one could combine the use of sublingual GTN with use of slow melt GSH tablets both of which could be dosed up to four times daily sublingually or buccally so as to approach continuous GTN dosing in cases of complicated angina. One could also consider including GSH in sublingual or buccal GTN formulations for increasing compliance.

For transdermal GTN dosing one could combine slow melt sublingual GSH and continuous transdermal GTN with L-arginine capsules. This based on a study which shows tolerance with continuous transdermal GTN is prevented by 700 mg L-arginine dosed 4 times/d [24]. In this approach angina patients would wear GTN patches continuously while using L-arginine oral capsules and sublingual GSH tablets 4 times daily. Including sublingual GSH in this regimen may add to the ability to control angina in its more severe (refractory) forms.

Conditions that May Benefit from GSH Based GTN Formulations

Three difficult to treat conditions that may benefit from GSH based GTN formulations are acute decompensated heart failure (ADHF), right heart failure following LVAD placement and refractory angina. All three of these conditions have been treated with GTN with results considered limited secondary to the development of tolerance with continuous sustained GTN administration. Therefore studies of the previously described GSH based GTN formulations are proposed in the discussion that follows.

Background for a Study of the Impact of IV GTN in 5% GSH on Diuretic

Resistance in Patients with Congestive Heart Failure

The background for this is a recent study of cimlanod, a new nitroxyl anion donor vasodilator being evaluated as a treatment for acute heart failure [27]. The purpose of the study was to assess the effect of cimlanod on urine output and sodium excretion in response to diuretic treatment in stable patients with HFrEF with EF $\leq$ 45%. This study found that, patients with HF, congestion and a reduced LVEF, the infusion of cimlanod reduced urine volume and sodium excretion, both before and after the administration of furosemide, accompanied by plasma volume expansion and an increase in total body water, findings seen with other vasodilators, to include nesiritide, serelaxin, minoxidil, nitroprusside and IV GTN in 5% dextrose, studied in heart failure patients. As a result it was concluded infusion of cimlanod attenuates a furosemide-induced diuresis in patients with heart failure and an LVEF $<45\%$, which might have adverse consequences for those who are already congested, i.e. ADHF patients. Also, that development of interventions for acute heart failure should include research focused on assessing effects on water balance and potential interactions with diuretic agents.

Rationale for a Study of the Impact of IV GTN in 5% GSH on Diuretic Resistance in Patients with Acute Heart Failure

Regarding the assessment of effects of vasodilators on water balance and interactions with diuretic agents, heart failure itself, unrelated to treatment, leads to increased renal superoxide levels via hypoperfusion, eNOS uncoupling, NADPH oxidase upregulation and NO/superoxide peroxynitrite formation [28]. Therefore, those vasodilators previously mentioned, to include cimlanod, that failed in previous clinical ADHF trials would either have had no benefit on diuretic responses or impaired responses leading to diuretic resistance (cimlanod).

As discussed previously [6], cimlanod is a nitroxyl anion donor and nitroxyl (HNO) reacts with molecular oxygen and forms peroxynitrite at physiological pH [29]. Therefore, one can view cimlanod as a peroxynitrite prodrug. Furthermore, peroxynitrite is a highly reactive radical which can uncouple eNOS by oxidizing BH₄ like superoxide from which it can be formed. All of this as discussed below explains cimlanod's effect to blunt Na⁺ and water excretion as in the above discussed study [27].

Mitochondrial superoxide has been found to reduce urine flow and sodium excretion in a rat kidney study [30]. And this was prevented using a superoxide dismutase mimetic. In so far as GSH similarly suppresses mitochondrial superoxide from GTN induced mitochondrial complex I uncoupling, it is proposed that IV GTN in 5% GSH be used in place of cimlanod in a repeat of the previously discussed cimlanod diuretic study [27]. This is to improve sodium excretion and diuretic responses thereby reducing or preventing diuretic resistance, in addition to preventing tolerance. Elimination of diuretic resistance and tolerance in the treatment of ADHF would be a significant improvement.

As for a reason to anticipate a positive response to IV GTN in 5% GSH, this is based on the positive effect seen with the SGLT inhibitors in acute heart failure. Similarly to GSH, dapagliflozin, a SGLT inhibitor, has been found to reduce oxidative stress in human proximal tubular cells [31]. This may explain the results of empagliflozin, another SGLT inhibitor, on the response to diuretics and urine output seen in the EMPAG-HF trial [32], all of which suggests a rationale for combining these two agents in the treatment of ADHF.

Treating Right Heart Failure Following Left Ventricular Assist Device Placement with IV GTN in 5% GSH

Similarly to CHF from left heart failure, RHF may be another disease with mitochondrial superoxide in its underlying pathogenesis. A recent RHF pulmonary artery banding study done in mice revealed the transition of maladaptive RV remodeling into RHF involved mitochondrial superoxide [33]. This may be process that can be blocked or reduced by GSH based GTN formulations used in the treatment of pulmonary hypertension.

In patients requiring LVADs for treatment of advanced HF, there is the additional problem of RHF from pulmonary hypertension induced by the operation of the device. RHF was seen in upwards of 20% of patients in the MOMENTUM-3 study. The use of an RVAD was required in 4% [34]. Treatment approaches for this problem include the use of diuretics, hemofiltration, inotropes, rhythm synchronization in addition to pulmonary vasodilators for reducing post capillary pulmonary hypertension.

Multiple approaches for treating pulmonary hypertension leading to RHF seen with LVADs have been tried with only short- term success. This includes inhaled nitric oxide, IV GTN in dextrose 5%, nitroprusside, inotropes, sildenafil and endothelin antagonists. The vasodilator most frequently used has been nitroprusside. As discussed previously [6], IV GTN in 5% GSH would be the next logical vasodilator that could be considered on the basis that superoxide from the use of GTN in dextrose 5% may have limited previous attempts to control RHF, or promoted RV remodeling.

For those patients who have responded favorably with extended use of LVADs there is a need to improve the process for explantation to reduce the need for heart transplantation. Currently up to five oral drugs are required to maintain patients hemodynamically stable during the weening process which is successful in about 50 % of attempts after 18 months [35]. Therefore this paper proposes studying the use of IV GTN in 5% GSH to reduce the need for multiple oral agents and to reduce weening time.

Treating Refractory Angina with Sublingual and Transdermal GTN Combined with Sublingual GSH

Refractory angina (RA) is conventionally defined as a chronic condition (≥ 3 months in duration) characterized by angina in the setting of coronary artery disease (CAD), which cannot be controlled by a combination of optimal medical therapy, angioplasty or bypass surgery, and where reversible myocardial ischaemia has been clinically established to be the cause of the symptoms [36]. And this condition is described by the term refractory because, until recently, multiple treatment approaches

have been unsuccessful. However it has very recently been shown in the ORBITA-COSMIC study that, via a mechanism unrelated to increased blood flow, a reduction in angina frequency beginning at 10 weeks post insertion may be achieved using a coronary sinus reducer (CSR) [37].

As for the mechanism explaining the benefit seen ORBITA-COSMIC one explanation consistent with the delayed onset of benefit beginning 10 weeks, is reduction in inflammation. There are no studies of inflammation in refractory angina but inflammation is thought to play a major pathological role in the in the severity of unstable angina [38]. If inflammation is involved, treatment with previously discussed sublingual and transdermal GSH based GTN approaches, following and in combination with CSR, may further improve results seen in the ORBITA-COSMIC study. This is because mitochondrial superoxide leads to activation of the NLRP3 inflammasome [39] and supplementing GSH is a way of preventing activation of the NLRP3 inflammasome which leads to vascular inflammation [40].

Summary and Conclusions

- 1]Uncoupled mitochondrial complex I from GTN mediated S-nitrosylation is the site of the first superoxide radical that leads to a cascade involving ALDH II and eNOS uncoupling, activation of PKC, activation of NADPH oxidase that ends in peroxynitrite formation with loss of vasodilator function and GTN tolerance.
- 2] baseline pre-treatment renal Na⁺ excretory function and urine output of heart failure patients is reduced secondary to the inhibitory effect of mitochondrial superoxide and related redox species on renal tubular function.
- 3] previous vasodilator drugs, which failed in the treatment of ADHF, failed because those agents did not suppress superoxide, and underlying renal tubule redox disturbances, or made it worse creating diuretic resistance as a result.
- 4] New GSH based GTN formulations and related treatment approaches, may, because of suppressing mitochondrial superoxide from GTN, and redox disturbances of diseases treated by GTN, improve the treatment of those diseases.

Abbreviations

ADHF	acute decompensated heart failure
AHF	acute heart failure
BH4	tetrahydrobiopterin
eNOS	endothelial nitric oxide synthase
GSH	glutathione
GTN	nitroglycerin, glycerol trinitrate
HF	heart failure
IV GTN/GSH	intravenous nitroglycerin in glutathione 5%
LVAD	left ventricular assist device
NAC	n-acetylcysteine
NO	nitric oxide
OONO	peroxynitrite
PKC	protein kinase C
RHF	right heart failure
ROS	reactive oxygen species
RNS	reactive nitrogen species
RVAD	right ventricular assist device
SGLT	sodium glucose transporter

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