Nitric Oxide Is the Cause of Nitroglycerin Tolerance: Providing an Old Dog New Tricks for Acute Heart Failure

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Abstract
Our paper highlights the past 50 years of research focusing solely on tolerance involving nitroglycerin (glyceryl trinitrate, GTN). It also identifies and discusses inconsistencies in previous mechanistic explanations that have failed to provide a way to administer GTN continuously, free of limitations from tolerance and without the requirement of a nitrate-free interval. We illustrate, for the first time in 135 years, a mechanism whereby nitric oxide, the mediator of vasodilation by GTN, may also be the cause of tolerance. Based on targeting superoxide from mitochondrial complex I, uncoupled by glutathione depletion in response to nitric oxide from GTN, a novel unit dose GTN formulation in glutathione for use as a continuous i.v. infusion has been proposed. We hypothesize that this will reduce or eliminate tolerance seen currently with i.v. GTN. Finally, to evaluate the new formulation we suggest future studies of this new formulation for the treatment of acute decompensated heart failure.

Keywords
nitric oxide, nitroglycerin tolerance, L-arginine, glutathione, superoxide, complex I, heart failure

Introduction
Tolerance to GTN is defined as the gradual loss of hemodynamic and antianginal activity during sustained therapy. It was first reported in 1888 by Stewart.1 Subsequently numerous theories have been proposed to explain tolerance. In 1995 it was demonstrated that tolerance involves superoxide and that removal of the endothelium improves tolerance in association with a reduction of superoxide levels in tolerant vessels.2 Soon thereafter mitochondria were shown to be another source of superoxide in mice made tolerant to GTN.3-5

Despite countless amounts of research funding over the past 50 years, none of the several mechanisms for explaining tolerance that were prominent prior to the superoxide hypothesis have led to a unit dose GTN formulation that can be administered continuously without tolerance. Non-specific eccentric nitrate dosing via nitrate free intervals was the only approach effective that entered everyday practice.

The scope of this paper is limited to GTN and tolerance resulting from superoxide and the use of i.v. GTN in the treatment acute decompensated heart failure (ADHF). This is because of both, the ever increasing significance of ADHF in the overall spectrum of heart failure as a cardiovascular disease burden, along with the expectation of benefit from eliminating tolerance from i.v. GTN in its treatment. This is particularly important now as that results of studies on treatment of ADHF with nesiritide, the recombinant form of the 32 amino acid human B-type natriuretic peptide6 and vasodilator relaxin7 were disappointing. Furthermore, tolerance which limits the use of GTN in treating angina has become less of a concern recently since other agents such as ranolazine and trimetazidine are available, thereby making ADHF the current area of unmet need.

Therefore, for an extensive and current discussion of the overall subject of nitrate tolerance the reader is referred to,8 which is focused on nitrates and nitric oxide (NO) donors other than GTN and indications other than ADHF, such as angina. In our paper (1) we discuss mechanisms that form the background for a cascade of superoxide production leading to tolerance and (2) highlight alternate approaches for targeting superoxide and its involvement in GTN tolerance. Next, (3) we describe the
studies questioning the role of sulphydryl (glutathione) depletion and the involvement of superoxide in tolerance. Finally, (4) we suggest a rationale for reformulating i.v. GTN in 5% glutathione (GSH) using 2% L-arginine as buffer and (5) propose clinical trials to evaluate the reformulation in the treatment of ADHF.

**Studies Illustrating the Involvement of Superoxide in GTN Tolerance**

**Mechanisms Involving GSH Dependency in GTN Vasodilation and Tolerance**

Approximately 50 years ago tolerance to GTN was proposed to be GSH dependent and was found to result from depletion of sulphydryl-groups with prolonged GTN treatment. This led to the “sulphydryl—depletion hypothesis.”

In 1987 Packer et al reported that tolerance to GTN, seen in the treatment of heart failure, can be prevented by the GSH prodrug N-acetylcysteine (NAC). This study was thought to be further evidence in support of the “sulphydryl—depletion” hypothesis and followed 2 earlier studies suggesting NAC prevents tolerance.

Subsequently, confirming Needleman’s initial observation, it was shown that the immediate vasodilator actions of GTN, prior to the development of tolerance, are potentiated by GSH when both, GTN and GSH, are mixed in-vivo by simultaneously administering the two into human coronary arteries.

More recently in the randomized, double blinded, placebo controlled NACIAM study, low dose i.v. GTN administered in combination with high dose i.v. NAC over a 2 day period was shown to reduce myocardial infarct size in comparison to i.v. GTN administered in combination with placebo. It was concluded that NAC potentiated the actions of GTN but the mechanism of potentiation was not addressed.

Tolerance induced by GTN has been shown to increase superoxide generation from mitochondrial complex I as a result of depletion of GSH. Furthermore, GSH was found to reverse superoxide generation and tolerance. These results have been further confirmed in complex I knock out studies.

As shown by Jekabson et al, NO and calcium uncouple complex I which results in increased superoxide production. Exogenously administered GSH suppresses this increase in superoxide. These data, plus data from others indicate that GSH is required for normal complex I coupling and that NO from GTN causes tolerance by uncoupling complex I.

**Mechanisms of Tolerance Resulting From Impaired GTN Bioconversion Due to Inactivation of ALDH 2 by ROS/RNS From Complex I**

The vasodilatory actions of GTN have generally been attributed to its bioconversion into the relaxant agent NO. The most recent evidence suggests a central role for mitochondrial aldehyde dehydrogenase (ALDH)-2 in GTN bioactivation. This process is thought to be subject to inactivation by reactive oxygen and nitrogen species (ROS and RNS) thereby leading to tolerance.

**Mechanisms Involving L-Arginine Dependency in GTN Vasodilation and Tolerance**

Altogether there are 7 studies showing L-arginine involvement in GTN tolerance. Two of these involve the use of L-arginine in clinical applications and 5 are in vitro studies.

**Clinical studies showing L-arginine dependency in the actions of GTN.**

In 2002 Parker et al showed the tolerance was prevented in patients with angina who were treated with transdermal GTN patches applied continuously and supplemented with 700 mg L-arginine capsules administered every 6 h. A report of 2 cases by Kaesemeyer et al showed prevention of tolerance in unstable angina and accelerated hypertension with i.v. GTN 200 µg/mL in 10% L-arginine used in place of and on top of, standard i.v. GTN 200 µg/mL 5% dextrose.

**In-vitro studies of L-arginine dependency in the actions of GTN.**

Endothelial nitric oxide synthase (eNOS) is a site of superoxide synthesis when endothelial cells are placed in L-arginine starved buffer and treated with GTN. This is because eNOS requires both L-arginine substrate and the cofactor BH4 at the site of its activity for endothelium-derived NO production (EDNO). Otherwise superoxide is formed in place of EDNO. Furthermore, both substrate and cofactor requirements are independent of, and persist, even when GSH levels are sufficient to prevent superoxide from mitochondrial complex I. Put another way, uncoupling of eNOS can occur under GSH dependent, GSH independent conditions and when both conditions are simultaneously present.

Adding to the foregoing studies, prolonged GTN treatment reduces tissue levels of L-arginine by 40%. This was associated with tolerance in aortic rings that was reversed by supplementation with L-arginine.

In a subsequent study involving GTN-treated rabbits, EDNO production in mesenteric artery was significantly reduced. This reduction in EDNO was suggested to be due to a limitation of L-arginine availability mediated by increased superoxide, the production of which is dependent on activation of an angiotensin II-PKC pathway.

**Cascading of Superoxide Mechanisms That Mediate GTN Tolerance**

In view of the foregoing studies, superoxide mediated GTN tolerance can be viewed as a 7 component process (Figure 1).

1. Beginning at complex I, NO from GTN either from denitration or eNOS or both, leads to inhibition of complex I as a result of calcium dependent depletion of GSH. This is followed by GSH suppressible superoxide generation by complex I. Afterward, GSH is oxidized to GSH disulfide (GSSG) which further depletes GSH and amplifies...
superoxide production. Superoxide subsequently combines with NO and peroxynitrite is formed along with other ROS and RNS.\(^{18,22,30}\)

2. ALDH-2 mediated bioconversion of GTN to NO is thought to be subject to inactivation by ROS and RNS from step I, thereby leading to a loss of NO from bioconversion.\(^{16,22,31}\)

3. RNS from ALDH-2 upregulate arginase II via activation of RhoA/Rho kinase and reduce L-arginine availability to eNOS.\(^{32}\)

4. EROS uncoupling from both, L-arginine and BH\(_4\) depletion further promotes superoxide production.\(^{33}\)

5. Superoxide from GTN activates PK C and PK C subsequently activates NADPH oxidase, a fourth site of superoxide.\(^{34}\)

6. Superoxide from all 4 sites induces formation of peroxynitrite and related radicals in place of NO from ALDH-2 and EDNO from eNOS.\(^{35,36}\)

7. Tolerance is the end result of loss of vasodilator function. EC indicates endothelial cells; ARG-2, arginase-2; BH\(_4\), 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).

The 7 step mechanism provides the answer to the long standing question—"what causes nitroglycerin tolerance?" As illustrated in Figure 2, NO, the mediator of vasodilation by GTN, may also be the cause of tolerance as a result of depleting mitochondrial GSH stores. We refer to this as the "Janus effect"
of GTN.” This explains the potentiation of GTN’s actions by GSH that was seen previously with the combined administration of GTN and GSH in human coronary arteries. Prevention by GSH of complex I uncoupling by NO from GTN, and eNOS uncoupling by L-arginine, avoids the cascading effect of superoxide depicted in Figure 1. This also protects ALDH-2 and eNOS as synergistic sites of NO production for augmented and sustained vasodilation.

**Approaches for Targeting Superoxide and Its Involvement in GTN Tolerance**

Unlike the multiple other remaining sites of superoxide production, complex I and eNOS represent the opportunity to reduce generation of superoxide and scavenge it thereby targeting both the underlying diseases and superoxide as their product. Targeting complex I and eNOS also prevents the cascading processes in Figure 1. All the remaining sites offer only scavenging as a means of targeting superoxide. This leaves the underlying processes generating superoxide abandoned.

Regarding targeting NADPH oxidase for preventing tolerance, a study in Wistar rats and mice revealed that both nitrate tolerance and endothelial dysfunction were prevented by targeting complex I, but only endothelial dysfunction, not tolerance, was prevented by targeting NADPH oxidase. Accordingly, it was concluded, that NADPH oxide may be a target for preventing GTN-induced endothelial dysfunction whereas complex I, as a target, would prevent both tolerance and endothelial dysfunction. And, as the authors of this study point out, “activation of the vascular NADPH oxidase seems to be driven by ROS/RNS derived from the mitochondrial respiratory chain.” This is shown in Figure 1 with NADPH activation driven by ROS/RNS derived from both the mitochondrial respiratory chain and eNOS, with PK C as an intermediate, at the end of the cascade. This further describes “crosstalk” discussed in.

Therefore, unlike eNOS from which superoxide and tolerance is both independent and dependent on complex I and GSH, superoxide from NADPH oxidase is solely dependent on complex I and GSH. In other words, NADPH oxidase is not an independent cause, but rather an effect of tolerance, as shown in Figure 1 and, thereby making it an unlikely target for preventing GTN tolerance. This may also explain the prevention of endothelial dysfunction, but not tolerance, by targeting NADPH oxidase in.

Also consistent with the foregoing is a recent study of “human NADPH knock outs,” i.e. patients with chronic granulomatous disease, caused by mutations in genes encoding the main components of the phagocyte NADPH oxidase (NOX2) which failed to show protection from coronary atherosclerosis in patients with multiple risk factors. Another study by Sibley et al. questions the significance of NADPH oxidase as a target of cardiovascular disease prevention and an independent target for preventing GTN tolerance. So, to maximally prevent GTN tolerance a better approach from a mechanistic point of view is to simultaneously target suppression of superoxide/ROS/RNS from both complex I and eNOS as depicted in Figure 1.

Interesting in the context of targeting mitochondrial GSH levels is \( \alpha \)-lipoic acid (\( \alpha \)-LPA). Consistent with the hypothesis of our paper, \( \alpha \)-LPA given with GTN upregulates mitochondrial GSH levels and thus protects complex I from uncoupling by NO from GTN denitration. At the same time it facilitates ongoing NO donation by GTN via preventing inhibition of ALDH-2 activity. This would explain prevention of tolerance in mice seen by Dudek et al. However, as will be discussed below, GTN in that study was dosed noncontinuously. This may have only resulted in pseudotolerance. Furthermore, cross-tolerance with acetylcholine was not assessed. Therefore, the question of availability of GSH to complex I after 72 h of continuous GTN dosing, and true vascular tolerance with \( \alpha \)-LPA given continuously, needs further study. Furthermore, the study by Dudek et al. overlooks tolerance from eNOS uncoupling described in steps 3 and 4 above. Suppressing superoxide from both, complex I and uncoupled eNOS requires both, GSH and L-arginine and would not occur just using \( \alpha \)-LPA.
Another mechanism involving superoxide and contributing to NO-induced tolerance not described above includes S-nitrosylation, the covalent modification of a protein cysteine thiol by an NO group to generate an S-nitrosothiol. Superoxide from GTN treatment can alter equilibrium between ROS and RNS and lead to conversion of L-cysteine to S-nitrosocysteine. Such nitrosation of cysteine residues is a posttranslational modification that regulates protein function. As pointed out above, mitochondrial complex I undergoes S-nitrosylation by NO with increased superoxide as a result. Likewise, prolonged use of GTN has been found to lead to tolerance by desensitizing soluble guanylyl cyclase. However, this mechanism is superoxide dependent. And suppressing the cascade of Figure 1 with GSH and L-arginine would also be expected to suppress its contribution to tolerance development.

Regarding S-nitrosylation and the uncoupling of complex I in step 1 of the cascade of Figure 1, S-nitrosylation of Cys39 of complex I was reported as a key mechanism of cardioprotection during ischemia/reperfusion, which significantly limits myocardial infarction. Likewise, it is shown that S-nitrosylation of complex I reduces mitochondrial ROS formation.

Subsequent studies, however, have found the protective effect of NO on complex I to be dose and time dependent, occurring at lower and lost at higher doses and lost with longer exposure times (days—tolerance vs minutes—ischemia/reperfusion) and the development of tolerance. Most likely this is due to depletion of the GSH pool with increased superoxide and peroxynitrite production not seen at lower levels of NO from the brief exposure times of ischemia/reperfusion. Furthermore, this increase in peroxynitrite would augment uncoupling of complex I leading to additional increases in mitochondrial ROS formation that would override the above mentioned protective effect.

**Studies Questioning the Sulphhydryl Depletion and Superoxide Hypotheses**

Recently, debate has arisen around the sulphhydryl depletion hypothesis and the mechanism of sulphhydryl donors in the potentiation of the vasodilator actions of GTN. This concerns the NACIAM study and an acute interaction of NAC with GTN suggested to be non-enzymatic and inconsistent with tolerance reversal. However, we feel this does not disprove Needleman’s finding that the actions of GTN are GSH dependent. NAC is well known to be an antioxidant and may have potentiated GTN by scavenging superoxide which resulted from ischemia. Or it could be both, scavenging and suppressing superoxide from complex I by supplying GSH as a prodrug to support denitration of GTN and EDNO from eNOS.

Another question that could be asked related to NACIAM concerns ischemia. Ischemia depletes GSH. So one could ask how GTN even works in the treatment of ischemia. This question, however, overlooks activation of eNOS by GTN and EDNO production (Figure 1). So, even if GSH was totally depleted, GTN would still produce vasodilation, at least acutely, until L-arginine was depleted. This further explains the benefit of L-arginine seen previously. Therefore, it appears Needleman’s “sulphydryl-depletion” hypothesis still holds, especially considering that glutathione is a thiol (“sulphydryl”) containing compound. The only thing missing from Needleman’s initial description of his hypothesis was the role of NO in “depletion.”

Finally, a study that has questioned the validity of the free radical hypothesis looked at patients with angina undergoing elective bypass surgery. Patients were randomized to low dose i.v. GTN (10 μg/min) for 24 h prior to surgery vs no i.v. GTN. Tolerance was studied in excised segments of internal mammary artery and saphenous vein segments. In the GTN group there was a 3-5 fold decrease in vascular responsiveness, absence of cross-tolerance to nitroprusside and calcium ionophore A23187. Bioconversion was impaired and an increase in superoxide from inhibition of superoxide dismutase failed to produce a further decrease in vascular responsiveness. As discussed by the authors, the study had limitations related to the use of a low GTN dose for a period shorter than 72 hours that likely only lead to the induction of pseudotolerance instead of true vascular tolerance.

Pseudotolerance is the result of volume and salt retention, as well as the stimulation of counter-regulatory mechanisms which may alter the baseline hemodynamics of a patient during nitrate therapy. Failure to see cross-tolerance to non-nitrate donors in the study by Sage et al was another finding consistent with pseudotolerance. This is represented in Figure 1 without the red “X,” when EDNO is still produced by eNOS. However, with a dose of GTN higher than that used by Sage et al and given for longer periods (up to 72 h) the “X” in our Figure 1 represents true vascular tolerance in which all eNOS is fully uncoupled.

**A Unit Dose GTN Formulation for Prolonged Continuous Tolerance-Free Intravenous Administration in the Treatment of ADHF**

As Figure 1 illustrates, the actions of GTN are dependent on both GSH and L-arginine. Based on prior studies, especially the study by Kugiyama et al, we have re-evaluated the current use of GTN.

The most commonly used form of GTN is GTN in 5% dextrose. This is a drug for intravenous administration and, among other uses, is part of guideline directed treatment of ADHF. However, its use is limited by tolerance. To overcome this limitation, we propose a reformulation of GTN in 5% GSH that is buffered with 2% L-arginine instead of GTN in 5% dextrose. We have found that 2% L-arginine maintains pH of GTN in 5% GSH between 4.0-4.1 (3.0-5.0) for well over a year (unpublished data). The differences between GTN in 5% dextrose and GTN in 5% GSH are illustrated in Table 1. Qualitatively, administration of both forms of i.v. GTN involves GTN, glucose, GSH and L-arginine. Quantitatively, due to the
absence of a dilutional effect of dextrose replaced by GSH, the impact on endogenous levels of glucose, GSH and L-arginine are anticipated to be much different as illustrated (Table 1).

I.v. GTN in GSH will target superoxide generation from both complex I and eNOS to diminish or eliminate tolerance from superoxide presently seen with GTN in dextrose. By replacing dextrose with GSH we are supplying GTN directly to complex I thereby avoiding previous issues involving NACs functioning as a GSH prodrug verses it’s being an antioxidant. Furthermore, replacing dextrose with GSH will avoid possible dilutional effects of dextrose on endogenous GSH and L-arginine levels. Exogenous L-arginine, in addition to its use as a buffer, also supplies substrate to eNOS to prevent its uncoupling. Therefore, this formulation change covers GSH dependent, GSH independent and both as causes of superoxide from eNOS, as well as superoxide from GSH dependent complex I uncoupling from NO.

As in the case of dextrose, both GSH and arginine are non-xenobiotic endogenous substances referred to as GRAS (generally regarded as safe) agents. Therefore, animal toxicology studies are likely unnecessary (Kaesemeyer, personal communication, 1998, US FDA) for commercializing GTN in 5% GSH buffered with 2% L-arginine (the formulation presented to the FDA in 1998 had dextrose replaced solely by L-arginine as described in, while GTN in 5% GSH buffered with 2% L-arginine is a second generation drug created in 2019).

Furthermore, the data from the study of intracoronary combined administration of GTN and GSH by Kugiyama et al reduces concerns for safety for i.v. administering a combination of these 2 agents. That is, if there were no safety issues with intracoronary administration, systemic administration should similarly be safe. So, there are preliminary data that will support use of the formulation change in applications described here.

Clinical Trials Proposed to Evaluate GTN in GSH in the Treatment of ADHF

As for initially evaluating i.v. GTN in 5% GSH for its impact on tolerance, we propose a worsening acute heart failure non-responder study. Patients seen in emergency rooms treated with standard i.v. GTN in 5% glucose who appear to be failing initial treatment because of tolerance would be switched to i.v. GTN in 5% GSH. ADHF in these patients could be from either decoupling of heart failure patients with preserved ejection fraction (HFpEF) or reduced ejection fraction (HFrEF). However, since HFpEF patients generally have uncontrolled hypertension and respond better to suppression of oxidative stress than HFrEF patients, they might provide a better comparison of the response of i.v. GTN in GSH vs i.v. GTN in dextrose.

In this switch the GTN dose would initially be the same as the dose before the change to GTN in GSH. Outcomes monitored would include blood pressure responses, relief of dyspnea and tolerability. Reversal of tolerance would be reflected by improved blood pressure control that includes the ability to reduce the initial GTN dose along with relief of congestion and the ability of patients to return home at the end of a 48 h “short stay” visits.

This initial pilot study with a small number of patients would be open label. If reversal of tolerance is suggested by the use of GTN in GSH the results of this small study would be used as preliminary data justifying a randomized study of GTN in GSH. This follow on study would assess the need for initial hospitalization and re-hospitalization at 30 days and longer.

A study, similar to, in which i.v. GTN in 5% dextrose would be replaced by i.v. GTN in GSH should be performed to compare the effects of GTN in GSH to that of a new nitroxyl donor Cimlanod on hemodynamic parameters of patients with heart failure. Cimlanod is a nitroxyl anion donor. Under aerobic conditions nitroxyl anion may form peroxynitrite, a toxic radical known to depress myocardial performance. Peroxynitrite formation may explain the absence of sustained hemodynamic effects on termination of Cimlanod infusions in ADHF patients. This may complicate the process of converting to oral agents, such as sacubitril/valsartan, following decongestion, prior to discharging patients home. Absence of sustained hemodynamic effects may put Cimlanod at a disadvantage to GTN in 5% GSH. A repeat of the previously described 48 h study would answer this question. Thus, at best, nitroxyl donors, like Cimlanod, are vasodilators that do not address the underlying redox issues of heart failure while at worst they may aggravate it being prodrugs for peroxynitrite.

A third application of i.v. GTN in 5% GSH would be for facilitating and accelerating the process of left ventricular assistant devices extraction post-myocardial infarction as

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**Table 1. Comparison of the Changes in Glucose, GSH and L-Arginine Contents Between 2 Different GTN Modes of Administration: GTN in 5% Dextrose and GTN in 5% GSH.**

|                      | Exogenous glucose administration | Endogenous glucose level changes | Exogenous GSH administration | Endogenous GSH level changes | Exogenous L-Arg administration | Endogenous L-Arg level changes |
|----------------------|---------------------------------|---------------------------------|------------------------------|------------------------------|---------------------------------|---------------------------------|
| 100-400 µg/mL GTN in 5% dextrose, i.v. | ↑↑                              | ↑                              | N/A                          | ↓↔                           | N/A                            | ↓↔                             |
| 100-400 µg/mL GTN in 5% GSH and 2% L-Arginine buffer, i.v. | N/A                             | ↓↔                             | ↑                            | ↑                            | ↑                              | ↑                              |

Abbreviation: N/A, not applicable.

*Replacement of dextrose by GSH is anticipated to eliminate a dilutional effect of dextrose and thus have beneficial effects on the endogenous levels of glucose, GSH and L-arginine.*
described in Birks et al.\textsuperscript{61} This process requires the use of up to 5 drugs to control elevated wedge pressure during the weaning process. A continuous infusion of i.v. GTN in GSH via a wearable pump may also limit left ventricular remodeling.

In all of the above we feel that our reformulation of i.v. GTN will permit tolerance-free continuous administration in both, the treatment and prevention of heart failure decompensation. Furthermore, in addition to GTN tolerance, this formulation will target redox imbalances found in heart failure patients\textsuperscript{62,63} which were unaddressed in recent large clinical trials whose results were disappointing.\textsuperscript{6,7}

**Summary and Conclusions**

This article addresses the 135 plus year old question “what causes nitroglycerin tolerance?” A thorough review highlighting the past 50 years of research on GTN tolerance leads to the conclusion that NO, the mediator of vasodilation by GTN, is also the cause of tolerance. We believe that NO, in addition to mediating vasodilation by GTN, also causes tolerance, primarily as a result of depleting mitochondrial stores of GSH. This secondarily leads superoxide production from uncoupling of complex I and activation of a cascade of mechanisms further amplifying superoxide production to the point that vasodilation ceases (tolerance). Therefore, we propose reformulating i.v. GTN replacing dextrose with GSH. To evaluate our new formulation we have suggested a number of studies in ADHF that will target redox imbalances found in heart failure patients\textsuperscript{62,63} as well as the cause of tolerance. We feel that our reformulation of i.v. GTN will overcome recent shortcomings seen with the use of newer agents that only target vasodilation.

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