Inorganic Nitrates

Qi for Heart Failure With Preserved Ejection Fraction?

Kavita Sharma, David A. Kass

In 1847, the chemist Ascanio Sobrero announced a way to make highly explosive compounds. Among them was nitroglycerin, which he made by heating cellulose in the presence of nitric acid to generate a substance he noted resembled light-yellow olive oil (he was Italian after all). Alfred Nobel subsequently figured out how to stabilize the explosive part, ultimately developing dynamite. This made him rich but reportedly also contrite over the impact his invention had on military operations, leading to his creating the Nobel Prizes as a sort of societal payback. Meanwhile, a Scottish physician, T. Lauder Brunton, was using amyl nitrite to treat angina, and when it was later realized that men with coronary disease working in nitroglycerin and dynamite factories had fewer angina episodes while at work but more over the weekend, its clinical use took off.[1] Nitroglycerin is still widely used to treat angina and organo-nitrates, such as isosorbide di-nitrate, and its active metabolite isosorbide mononitrate (ISMN) with longer half-lives are also in clinical use.

Article, see p 1151

This is recent history compared with what the Chinese uncovered more than a millennium earlier. They found medicinal value in salpeter (KNO_3), an inorganic salt that dissociates in aqueous solution into nitrate anion. As explained in a text attributed to the fifth to sixth century Daoist alchemist and physician Tao Hongjing (Figure), the powder placed under the tongue caused heart *qi* (figuratively life force) to flow freely and treat chest pain and other conditions of cardiovascular distress.[2] In addition to its medical and explosive utility, salpeter was used by the middle ages as a food preservative. The element shared by organo-nitrates, such as nitroglycerin and inorganic KNO_3^-, is nitrate (NO_3^-). Nitrate itself has no physiological effects, but must undergo a 3-electron reduction to nitric oxide (NO) to be active, with nitrite (NO_2^-) as an intermediate compound. Dietary nitrate is found in green leafy vegetables and beetroot, in particular, and is absorbed through the gut without conversion. Approximately 25% of absorbed NO_3^- is converted to NO_2^- in saliva; the rest is excreted by the kidney.[3] Organo-nitrates are pharmaceuticals, with between 1 and 4 attached nitrate moieties, and are metabolized differently,[1] with high first-pass clearance in the liver and requiring P450 cytochrome oxidase or aldehyde dehydrogenase to generate NO.

Nitrates would seem the perfect therapy for heart failure because they induce arterial and venous vasodilation to decongest the central vasculature while reducing systolic workload. This includes their effects on large arteries to enhance systemic vascular compliance and on delaying the return of peripheral artery reflected waves to reduce pulsatile systolic afterload imposed on the heart.[4] NO also improves mitochondrial and, in turn, skeletal muscle energetic efficiency; it modifies proteins to enhance their function or protect them against oxidative damage; and by activating cyclic guanosine monophosphate (cGMP) and, thus, protein kinase G (PKG), it triggers multiple protective cardiac and vascular signaling events. As NO_2^- to NO conversion is enhanced in acidic environments, it is particularly potent in ischemic tissue in the heart and peripheral circulation, certainly relevant to heart failure pathophysiology.

Benefits of afterload reduction were studied with organo-nitrates, but their story quickly became complicated by their capacity to stimulate oxidative stress and, with this, develop tolerance.[1] Oxidative stress impairs NO signaling by reducing intrinsic NO synthesis and, thereby, its activation of key distal effectors—soluble guanylate cyclase to generate cGMP and, in turn, protein kinase G.[1] This ultimately limited the chronic use of organo-nitrates for heart failure. Inorganic nitrates and nitrites, by contrast, reportedly do not induce tolerance because their activation chemistry differ.[3]

A clinical population attracting particular attention for the therapeutic use of inorganic nitrates and nitrites is heart failure with a preserved ejection fraction (HFpEF). This syndrome, reflecting half of all heart failure patients, has traditionally presented with hypertension coupled to left ventricular hypertrophy and diastolic dysfunction. Indeed, for a long time, it was referred to as diastolic heart failure, and this term is still encountered. Increasingly, however, patients and the clinical studies they are recruited into have far less hypertension; half or less display left ventricular hypertrophy. Instead, we are seeing a high prevalence of other comorbidities, including obesity, diabetes mellitus, pulmonary hypertension, and skeletal muscle dysfunction.[5] To date, there is no approved evidence-based therapy for HFpEF. After a long run of negative trials that targeted the renin/angiotensin/aldosterone pathway, recent studies have examined NO–cGMP–PKG signaling. To date, this has not been auspicious. Studies of sildenafil, a phosphodiesterase-type 5 inhibitor,[6] ISMN,[7] and vericiguat, a soluble guanylate

From the Division of Cardiology, Department of Medicine and Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD.

Correspondence to David A. Kass, Johns Hopkins University, Cardiology, Ross 858, 720 Rutland Ave, Baltimore, MD 21205. E-mail dkass@jhmi.edu

(*Circ Res*. 2017;120:1057-1059.
DOI: 10.1161/CIRCRESAHA.117.310699.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.117.310699

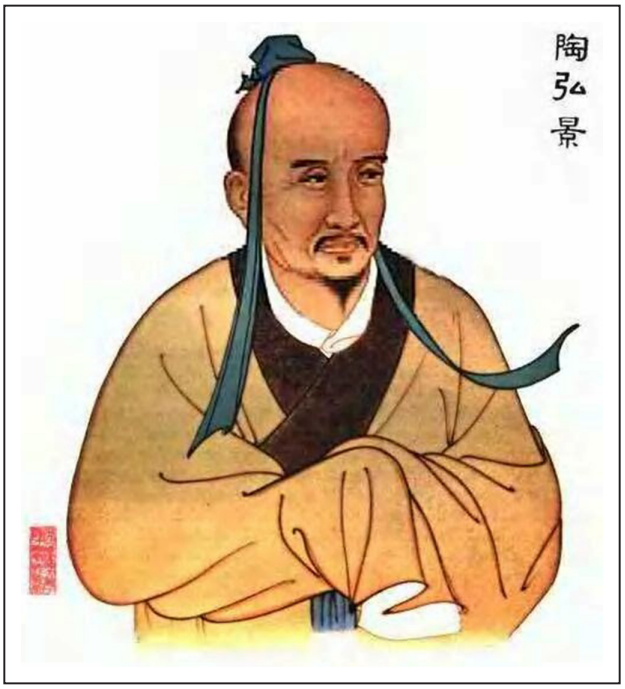

Figure. Tao Hongjing who in the fifth century described the use of saltpeter (KNO_3) for the treatment of cardiovascular disease.

cyclase stimulator,[8] all missed their primary end points. Sildenafil effects on secondary variables were mostly neutral, ISMN actually reduced daily activity levels and patient well-being, and vericiguat did not reduce left atrial size or NT-proBNP (N-terminal pro-B-type natriuretic peptide) levels (its primary end points), though some signal for symptom improvement at the highest dose was found. Yet another approach is currently being tested, combining the angiotensin receptor blocker valsartan with the neprilysin inhibitor sacubitril (LCZ696). It is thought that sacubitril can augment natriuretic peptide levels that would otherwise be catabolized by neprilysin, so cGMP/PKG signaling is on trial again.

Enter inorganic nitrates (and nitrites). Unlike organic nitrates, these substances have been shown to be more potent in states of hypoxia and acidosis, thus, more effectively delivering NO in times of need. Of the various formulations of inorganic nitrates, perhaps the most well-known is beetroot juice, a concentrated source of NO_3^-. In fact, the use of beetroot juice dates back to the Middle Ages when it was used to nullify the pungent effect of garlic on the breath. Other practical applications included its use as a coloring agent of wine and foods (it also turns urine and stool reddish), and eventually, its cardiovascular benefits were appreciated. Beetroot juice lowers blood pressure in animals and in humans[9] and is sold commercially as an exercise enhancer, improving endurance in athletes. It is widely available in supermarkets, online, and brick-and-mortar stores and marketed as a superfood to improve energy, stamina, and heart health.

In 2015, Zamani et al[10] studied the effects of a single dose of beetroot juice in HFpEF patients. They found that it improved exercise capacity by principally augmenting arterial vasodilation and associated with this, cardiac output, and exercise capacity. Tissue oxygen saturation index did not change. This result is interesting in light of earlier studies showing deficient arterial vasodilation as a primary noncardiac cause for reduced cardiac output and limited exercise reserve in HFpEF versus well-matched nonfailing controls.[11] Eggebeen et al[12] tested 1 week of beetroot juice therapy and found improved submaximal aerobic capacity in HFpEF subjects after 1 week of daily beetroot juice concentrate consumption. Sodium nitrite—the NO_3^- metabolite one step closer to NO—also has hemodynamic benefits in HFpEF patients, improving cardiac hemodynamics (eg, reducing exercise-induced rise in pulmonary capillary wedge pressure) and enhancing exercise performance.[13]

Beetroot juice has many other components besides NO_3^-, so a study of the ancient Chinese medicine—KNO_3—was in order. In a new phase IIa study, the same group now reports that 2 weeks treatment of HFpEF patients with this pure inorganic nitrate also has ameliorative impact on exercise performance in HFpEF patients.[14] The study is small (9 patients), primarily designed as a safety study, but with this, the investigators obtained a large amount of other data. Interestingly, neither of the primary findings from their prior beetroot juice study were recapitulated here, in that maximal oxygen consumption and systemic arterial vasodilation were not enhanced by KNO_3 treatment. However, both total work performed on the bike test and associated oxygen uptake were increased, and this was notable during the second half of the exercise period. Cardiac output (and stroke volume) reserve with exercise was unaltered by the treatment, and diastolic heart function and multiple blood pressure analytics were also unaffected. KNO_3 was well tolerated.

The study shows an apparent benefit on integrated exercise capacity, but this comes with caveats. The study was not placebo controlled, it was single blinded, and one must always be careful with such a design when doing exercise protocols because they are known to be sensitive to encouragement bias. The study was small and, thus, inadequately powered to make conclusions regarding many of the measurements presented. Trends or borderline differences in some variables could reflect type 1 or type 2 errors. The patient group was somewhat different from the group's prior study—being 30% black at most, whereas the prior study was 80% black ($P<0.015$ by χ^2).[10] This could be important, as isosorbide dinitrate combined with hydralazine is uniquely approved for blacks with heart failure with reduced ejection fraction because the role of nitric oxide signaling disease in this population differs from that in white.[15] Furthermore, HFpEF is increasingly being recognized in black populations, and their disease may require somewhat different therapy.

The authors theorized that the improved performance at higher levels of exertion might reflect better peripheral conversion of NO_3^- to NO because of more acidosis at moderate to severe intensity and a role of type 2 fibers in this conversion. HFpEF patients have indeed been found to have a relative decline in oxidative type 1 fibers,[5] reducing their exercise capacity. Perhaps, NO_3^- can leverage this to an extent. However, the lack of vasodilator reserve disparities or other evidence supporting improved peripheral perfusion (change in oxygen saturation or arterial-venous O_2 difference) counts against that. A more robust and adequately powered study is needed to determine whether this is a real reproducible benefit and why it is happening.

The authors mentioned the recent randomized trial of ISMN and HFpEF and its negative findings.[7] The patient population for this prior multicenter study was mostly white, about half male, and did not assess if therapy had demonstrable subacute benefits on exercise that then disappeared with chronic treatment. KNO_3 should have less tolerance, and Zamani et al[14] note this in their discussion. However, the ISMN results were potentially related to side effects from NO-dependent effects on blood pressure in HFpEF, and if so, then it becomes less clear how KNO_3 will avoid this.

Another aspect of this study worth noting is that the investigators screened 703 subjects to yield ultimately 12 for the trial. This is 1.7% of the screened group and likely a reflection of the enrollment criteria that excluded common comorbidities, such as atrial arrhythmias and general impediments to exercise. We are increasingly finding that HFpEF is less a disease, but rather more a syndrome of multiple comorbidities that include the heart but are not necessarily driven by the heart in all patients. Efforts are underway to better phenotype this patient population to target enrollment in clinical trials more effectively. Ultimately, larger-scale studies are needed to better understand the effects of nitrate/nitrite therapy in HFpEF and equally importantly the intolerances or adverse effects we may see down the road. There are 2 ongoing phase 2b randomized controlled trials testing the effects of inhaled nitrite (INDIE-HFpEF [Inorganic Nitrite Delivery to Improve Exercise Capacity in HFpEF]) and oral nitrites (KNO3CK OUT HFpEF [Effect of KNO3 Compared to KCl on Oxygen Uptake in Heart Failure With Preserved Ejection Fraction]) on exercise capacity, physical activity, and quality of life in HFpEF.

HFpEF continues to be among the largest unmet medical needs in Westernized societies. Any and all creative and effective approaches are encouraged, and the present work is a step in this direction. Whether this will end with beetroot juice, KNO_3, or nitrite salts that also have hemodynamic benefits coupled with better exercise duration in HFpEF all remain to be seen. If it happens with KNO_3, however, we can be sure Tao Hongjing will be smiling as we perhaps move a step closer to finding the *qi* in HFpEF.

Sources of Funding

This study was supported by American Heart Association Go Red for Women Network Grant: 16SFRN27870000 (D.A. Kass and K. Sharma) and R35-HL135827 (D.A. Kass).

Disclosures

None.

References

1. Daiber A, Münzel T. Organic nitrate therapy, nitrate tolerance, and nitrate-induced endothelial dysfunction: emphasis on redox biology and oxidative stress. *Antioxid Redox Signal*. 2015;23:899–942. doi: 10.1089/ars.2015.6376.
2. Butler AR, Moffett J. A treatment for cardiovascular dysfunction in a dunhuang medical manuscript. In: Lo V, Cullen C, eds. *Medieval Chinese Medicine: The Dunhaung Medical Manuscripts*. Abingdon: Oxon Routledge; 2104:363–368.
3. Chirinos JA, Zamani P. The nitrate-nitrite-NO pathway and its implications for heart failure and preserved ejection fraction. *Curr Heart Fail Rep*. 2016;13:47–59. doi: 10.1007/s11897-016-0277-9.
4. Pauca AL, Kon ND, O'Rourke MF. Benefit of glyceryl trinitrate on arterial stiffness is directly due to effects on peripheral arteries. *Heart*. 2005;91:1428–1432. doi: 10.1136/hrt.2004.057356.
5. Sharma K, Kass DA. Heart failure with preserved ejection fraction: mechanisms, clinical features, and therapies. *Circ Res*. 2014;115:79–96. doi: 10.1161/CIRCRESAHA.115.302922.
6. Redfield MM, Chen HH, Borlaug BA, et al; RELAX Trial. Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction: a randomized clinical trial. *JAMA*. 2013;309:1268–1277. doi: 10.1001/jama.2013.2024.
7. Redfield MM, Anstrom KJ, Levine JA, et al.; NHLBI Heart Failure Clinical Research Network. Isosorbide Mononitrate in Heart Failure with Preserved Ejection Fraction. *N Engl J Med*. 2015;373:2314–2324. doi: 10.1056/NEJMoa1510774.
8. Pieske B, Maggioni AP, Lam CK, Pietro MA, Ponikowski P, Shah S, et al. Vericiguat in patients with worsening chronic heart failure and preserved ejection fraction. Results of the soluble guanylate cyclase stimulator in heart failure patientss with preserved ef (socrates-preserved) study. *Eur Heart J*. 2017.
9. Siervo M, Lara J, Ogbonmwan I, Mathers JC. Inorganic nitrate and beetroot juice supplementation reduces blood pressure in adults: a systematic review and meta-analysis. *J Nutr*. 2013;143:818–826. doi: 10.3945/jn.112.170233.
10. Zamani P, Rawat D, Shiva-Kumar P, Geraci S, Bhuva R, Konda P, Doulias PT, Ischiropoulos H, Townsend RR, Margulies KB, Cappola TP, Poole DC, Chirinos JA. Effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction. *Circulation*. 2015;131:371–380; discussion 380. doi: 10.1161/CIRCULATIONAHA.114.012957.
11. Borlaug BA, Melenovsky V, Russell SD, Kessler K, Pacak K, Becker LC, Kass DA. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. *Circulation*. 2006;114:2138–2147. doi: 10.1161/CIRCULATIONAHA.106.632745.
12. Eggebeen J, Kim-Shapiro DB, Haykowsky M, Morgan TM, Basu S, Brubaker P, Rejeski J, Kitzman DW. One week of daily dosing with beetroot juice improves submaximal endurance and blood pressure in older patients with heart failure and preserved ejection fraction. *JACC Heart Fail*. 2016;4:428–437. doi: 10.1016/j.jchf.2015.12.013.
13. Borlaug BA, Koepp KE, Melenovsky V. Sodium nitrite improves exercise hemodynamics and ventricular performance in heart failure with preserved ejection fraction. *J Am Coll Cardiol*. 2015;66:1672–1682. doi: 10.1016/j.jacc.2015.07.067.
14. Zamani P, Tan V, Soto-Calderon H, et al. Pharmacokinetics and pharmacodynamics of inorganic nitrate in heart failure with preserved ejection fraction. *Cir Res*. 2017;120:1151–1161. doi: 10.1161/CIRCRESAHA.116.309832.
15. Stein CM, Lang CC, Nelson R, Brown M, Wood AJ. Vasodilation in black Americans: attenuated nitric oxide-mediated responses. *Clin Pharmacol Ther*. 1997;62:436–443. doi: 10.1016/S0009-9236(97)90122-3.

KEY WORDS: Editorials ■ heart failure ■ human ■ pharmacology ■ nitrate ■ nitric oxide

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

Inorganic Nitrates: Qi for Heart Failure With Preserved Ejection Fraction?

Kavita Sharma and David A. Kass

Circ Res. 2017;120:1057-1059
doi: 10.1161/CIRCRESAHA.117.310699
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571