SPECIAL REPORT

Nitroglycerin and Nitric Oxide — A Rondo of Themes in Cardiovascular Therapeutics

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The rondo is a musical form in which a single recurrent theme alternates with different melodic motifs that are each distinct from one another. The recurrent theme is typically repeated multiple times, providing both the overall unity for the musical movement and a familiar musical destination to which the piece returns after the presentation of distinct interspersed themes. The parallels between the rondo form and the cyclic temporal pattern of experimental therapeutics are uncanny. Clinical observation is a recurring theme in drug development, in which the effects of treatment observed in patients may suggest novel therapeutic indications (or unanticipated side effects) that lead to the identification of new molecular targets and pathways. These observations in patients are typically interspersed with increasingly sophisticated analyses in novel experimental models, yielding a kind of "drug-development rondo." The rondo being reviewed in this article takes place in the key of E — for endothelium. The drug in question is nitroglycerin, and its major active principal is nitric oxide, a gaseous molecule made in the vascular endothelium (and in other tissues) that is essential for vascular homeostasis.

The opening theme of our drug-development rondo can be traced back at least to the year 1768, when William Heberden coined the term "angina pectoris" and then published an evocative description of the symptoms of angina, which concluded with this discouraging admission: "With respect to the treatment of this complaint, I have little or nothing to advance." Just 6 years later, the English chemist and theologian Joseph Priestley discovered nitric oxide. However, these two coincidental discoveries would remain isolated, and a century would pass before nitroglycerin and related organic nitrate drugs would be applied to the effective treatment of angina. Yet another century would go by before the connections among nitric oxide, nitroglycerin, and angina pectoris were revealed — both in the laboratory and in the clinic. Discoveries with regard to the physical properties of gases moved forward apace, while the clinical characteristics of angina pectoris were being systematically explored, with no notion that these seemingly distinct spheres of inquiry would ever intersect.

The next new theme has its origins in organic chemistry. In 1846, the Italian chemist Ascanio Sobrero became the first person to synthesize nitroglycerin, the explosive properties of which were immediately appreciated. Like most good chemists of this bygone era, Sobrero then tasted his newly synthesized compound and noted the profound headache that ensued, a phenomenon quickly attributed to cerebral vasodilation. This simple clinical observation — that nitroglycerin dilates the vasculature — sparked a century-long dialogue between clinical pharmacologists and basic vascular physiologists, a dialogue that enabled many of the discoveries that are essential to our current understanding of the biologic functions of nitric oxide. This basic scientific advance led quickly to a return to the theme of clinical observation, and within just two decades of Sobrero's discovery, the vasodilatory effects of nitroglycerin and the related compound amyl nitrate were exploited for therapeutic effect. By the 1860s, the British pharmacologists and physicians T. Lauder Brunton and William Murrell were using nitrate compounds to treat patients with angina and hypertension, and use of the drugs quickly became widespread.[1] Indeed, the industrialist (and later philanthropist) Alfred Nobel had debilitating symptoms of angina pectoris that were ameliorated by nitroglycerin. Nobel had made his fortune after inventing a nitroglycerin detonator that permitted control of this highly explosive compound and facilitated the use of nitroglycerin in munitions. Nobel observed that his use of nitroglycerin to treat his own symptoms of angina reflected an "irony of fate." The huge gulf between the destructive potential of nitroglyc-

erin and its therapeutic applications provides an extreme example of what has now become known in the pharmaceutical industry as "off-target" chemical properties.

The next thematic development involved a return to the laboratory, this time in an attempt to understand how nitroglycerin exerts its effect on blood vessels. During the first half of the 20th century, prompted by the notion that nitroglycerin might mimic an endogenous molecule, pharmacologists devoted considerable effort to the characterization of the physiological responses of various tissues to nitrates.[2] They found that nitroglycerin and related chemicals not only promoted the relaxation of vascular tissues but also exerted a relaxing effect on tracheal and gastrointestinal smooth-muscle tissues. Indeed, it seemed as if physiologists could relax virtually any smooth-muscle–bearing tissue simply by pouring on the nitroglycerin. Inspired by these laboratory observations, pharmacologists expanded the clinical applications of nitrates beyond angina to the treatment of reactive airway disease and gastrointestinal sphincter spasms. As nitrates entered more widespread clinical use, a second important clinical phenomenon was observed: patients who received long-term treatment with nitrates began to show tolerance for the drug, requiring larger doses over time to achieve the same therapeutic effect. In addition, there was anecdotal evidence that people who worked with dynamite, which involved handling nitroglycerin during the workweek, were having "Sunday heart attacks." During the weekend, when workers were not exposed to nitroglycerin, some had rebound arterial vasoconstriction, leading to myocardial infarction. These clinical observations were reminiscent of those regarding drugs such as opiates, which activate physiological signaling systems, and suggested that somehow nitroglycerin was also mimicking the action of an as yet unknown endogenous biomolecule. It was time to return to the bench.

A century would pass between the first use of nitroglycerin in the treatment of angina pectoris and the discovery of the endogenous vascular signaling system that was the molecular target of this treatment. In 1977, Ferid Murad demonstrated that nitroglycerin and related compounds were in fact prodrugs and that the biologically active molecule released from these compounds was the free-radical gas nitric oxide. Furthermore, Murad and colleagues discovered that the relaxing effect nitric oxide had on vascular smooth muscle was mediated by enzymatic machinery analogous to the recently discovered beta-adrenergic receptor signaling cascade. The difference was that nitroglycerin treatment promoted an increase in the second messenger molecule cyclic guanosine monophosphate (cGMP), in contrast with the increase in the similar but distinct messenger molecule cyclic adenosine monophosphate, which had been previously observed after beta-adrenergic stimulation. Murad's elucidation of the central role nitric oxide played in the vasodilatory effects of nitrates provided a basic scientific explanation for a long-standing clinical question in pharmacology, but it also raised the question of whether nitric oxide might also have a role in normal vascular physiologic processes. It seemed highly unlikely that nature would engineer such an elegant signaling cascade only for the purpose of its activation by a drug that would not be discovered until the 19th century.

Just a few years after Murad discovered the vasodilatory effect of nitric oxide, Robert Furchgott made the serendipitous observation that intact blood vessels would relax in response to acetylcholine only if they possessed an intact endothelial lining. Furchgott's observation led to his proposal that the endothelium produced an unknown substance that induced relaxation of the underlying smooth muscle, and he called the substance endothelium-derived relaxing factor (EDRF). Soon after Furchgott made this observation, the field returned to the rondo of clinical observation, as the connection was made between EDRF and human disease: coronary atherosclerosis. Normal human coronary arteries dilate in response to acetylcholine (as Furchgott had shown in ex vivo arterial preparations), but, paradoxically, atherosclerotic vessels constrict.[3] The discovery of a connection between coronary atherosclerosis and abnormal vasomotion fueled the already feverish hunt for the identity of EDRF. In 1987, Salvador Moncada and Louis Ignarro independently discovered that EDRF was in fact nitric oxide — the same molecule that had been unwittingly exploited as a therapeutic agent during the previous century. Given the realization that the endothelium could synthesize the nitric oxide, scientists in the subsequent decade witnessed the discovery of the enzymatic

machinery responsible for its in vivo synthesis. The endothelial isoform of nitric oxide synthase was discovered and cloned in 1992. Thus, there was now a complete physiological context for the function of the archetypal drug nitroglycerin and related organic nitrate vasodilators. Nitroglycerin and its relatives are metabolized to yield nitric oxide, an endogenous endothelium-derived molecule that is normally synthesized in the vascular wall by endothelial nitric oxide synthase and is a critical determinant of vascular tone. The discovery of endothelial nitric oxide synthase in other tissues,[4] notably cardiac myocytes,[5] helped to establish broad physiological roles for endothelial nitric oxide synthase and nitric oxide that extended well beyond the vascular wall. With its importance in physiology now established, nitric oxide was named "Molecule of the Year" by the journal *Science* in 1992,[6] and shortly thereafter, endogenous enzymatic pathways that convert nitroglycerin to nitric oxide were elucidated.[7] For their seminal work "concerning nitric oxide as a signaling molecule in the cardiovascular system," Furchgott, Ignarro, and Murad were awarded the Nobel Prize in Physiology or Medicine in 1998.

Coming full circle to the theme of clinical observation and basic discovery that marked the beginning of the centuries-long, interwoven stories of angina, nitroglycerin, and nitric oxide, the understanding of the cellular and biochemical role of nitric oxide in regulating vascular tone fueled a new burst of drug development. For example, inhaled nitric oxide has proved to be highly effective in treating pulmonary hypertension. By delivering nitric oxide directly to the desired site of action (i.e., the pulmonary arterioles), inhaled nitric oxide can preferentially dilate the pulmonary vasculature while minimizing the effects on systemic arterial tone.[8] The discovery that nitroglycerin (by means of its nitric oxide content) increases intracellular cGMP levels led to efforts to formulate novel antianginal agents that enhance cGMP-modulated signaling pathways. Cyclic nucleotides are degraded by a family of phosphodiesterases that can attenuate the signaling cascade activated by nitric oxide by catabolizing cGMP. The archetypal cGMP phosphodiesterase inhibitor sildenafil was originally designed as an antianginal drug and was then found to be an effective treatment for erectile dysfunction. The path from laboratory to clinic has been characterized as "bench to bedside," but the development of sildenafil as an antianginal agent on the basis of its biochemical properties may represent the first route to drug discovery that can be described as "bench to

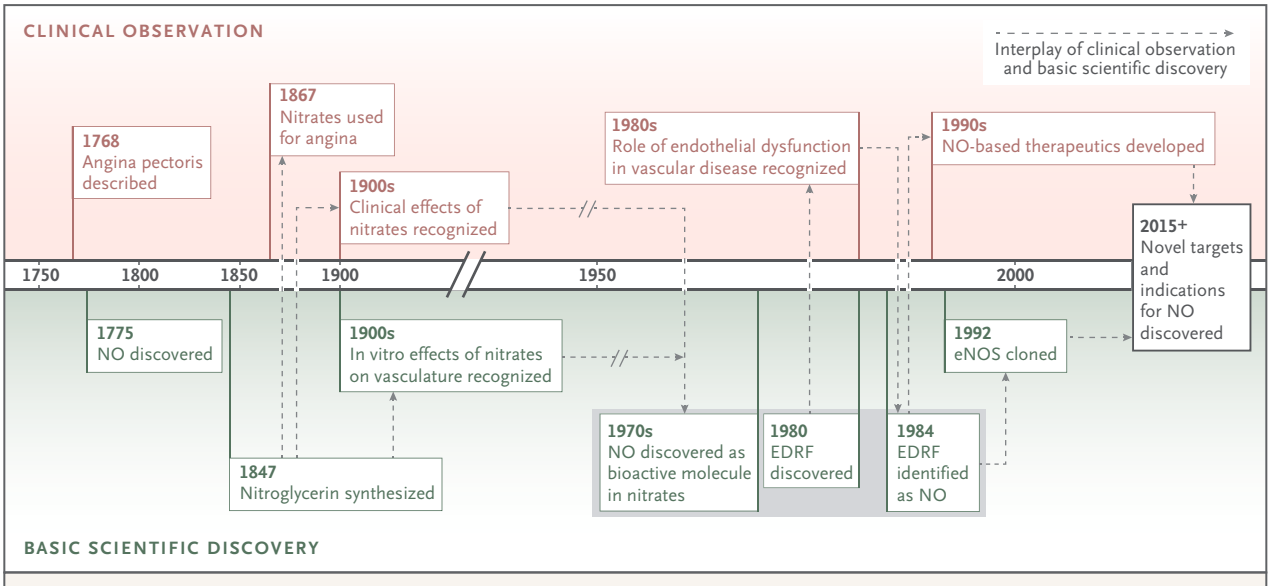

Figure 1. The Rondo between Clinical Observations and Scientific Discoveries in the Endothelium.

The timeline depicts the interplay between clinical observations and basic scientific discoveries involving nitroglycerin, nitric oxide (NO), and endothelial signaling pathways. EDRF denotes endothelium-derived relaxing factor, and eNOS endothelial isoform of nitric oxide synthase.

bedside to bedroom." Since deranged intracellular nitric oxide signaling plays a central role in the pathophysiological features of many other disease states, phosphodiesterase type 5 inhibitors continue to be explored as novel therapeutic agents for heart failure and pulmonary hypertension, among many other diseases. Other novel aspects of the chemistry of nitric oxide, such as its ability to modify cysteinyl groups post-translationally to form *S*-nitrosothiols,[9] are being applied and exploited in new therapeutic contexts ranging from neurodegeneration to cardioprotection. During this explosion of therapeutic agents based on nitric oxide, basic laboratory investigation has also flourished, finding a key role for nitric oxide in the modulation of mitochondrial function.[10] Similarly, basic investigation has continued to push the frontiers of the physiologic role of nitric oxide by revealing new endogenous sources, such as its formation by the chemical reduction of nitrite.[11]

As our understanding of the pathways that link the chemical properties of nitroglycerin to its therapeutic roles has evolved, we can discern recurring themes that involve the interplay of basic discovery and clinical observation. Figure 1 shows the interplay between clinical observation and scientific discovery over time as they relate to nitroglycerin, nitric oxide, and endothelial signaling pathways. Today, nitric oxide stands at the center of our understanding of the pathobiology and treatment of many diseases in organ systems ranging from the cardiovascular system to the gastrointestinal tract to the brain. However, had it not been for the fact that each clinical observation spurred new scientific lines of inquiry, our understanding of the physiologic and pathophysiologic roles played by nitric oxide would be far less complete. The development and refinement of organic nitrate-based vasodilators reflects the recurrent interplay between the experimentalist's bench and the clinician's search for effective new therapies. This symbiotic exchange between clinical practice and basic science has enabled us to achieve a much richer understanding of the origin and treatment of disease than either field is likely to have allowed in isolation, highlighting the importance of the flow of information from the bedside back to the bench. Scores of clinical trials are currently under way, inspired by new basic knowledge of the pharmacologic properties of nitroglycerin and the chemical properties of nitric oxide, ranging from studies on the use of nitroglycerin to enhance the delivery of drugs to tumors to trials of novel drugs for heart failure that target guanylate cyclase (see several examples at ClinicalTrials.gov [https://clinicaltrials.gov/ct2/results?term=nitroglycerin&recr=Open]). If the past century is any indication, new themes for the rondo of endothelial biology and the therapeutic applications of nitric oxide and nitroglycerin remain to be written.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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