



What the history of nitric oxide study can tell us about translational research?

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Abstract: Innovation is one of the critical elements in scientific research. Nitric oxide (NO) was once considered as a toxic molecule and mocked by Alfred Nobel, the founder of the Nobel Prizes, for using nitroglycerin, the producer of NO, has nowadays become an effective therapy for pulmonary hypertension in adults and infants. Three scientists won Nobel prize due to their astonishing discovery of the effects of NO on vasculature. In this article we review the dramatic history of NO which inspires the researchers to transform thinking pattern and translate the lab results to clinical applications.

Key words: translational medicine; innovation; nitric oxide

Scientific research topic selection has always been quite a puzzle for probably every researcher. As we all know that a valuable scientific subject which finally could translate into clinical application is frequently innovative at first. However, the breakthrough point is so difficult to find out, especially compared with the apparent scientific problems it behind.

The breakthrough from zero to one is innovation, but the steps from one to two, three, and four.....each step is also innovation. Therefore, how to innovate with the published scientific achievements? How to explore from the superficial to the systematic layers? How to break thought einstellung and think about research outside of our familiar speciality? Maybe the history of nitric oxide (NO) studies would answer the questions.

From toxic molecule to vasodilation factor

NO was widely known as a toxic molecule, one of the environmental pollutants found in smog and cigarette smoke. Both United Kingdom and United States limited the maximum concentration of NO as 25ppm in industrial area. Since a woman of 39 years old dead from the contamination with NO in a nitrous oxide cylinder during

the anesthesia procedure and another woman of 24 years old poisoned for the same reason in 1966 at the General Hospital, Bristol^[1], the role of the molecule became more horrible than before. The dramatic change happened to the recognition of NO began with the discovery of the vasodilation function. An editorial named "NO news is good news" published in *Science* and proclaimed NO as "Molecule of the Year" in 1992 due to some exciting and startling discoveries of the role of NO in vasodilation effects^[2]. Researchers from the University of California found that the infusion of NO could relax the constricted bovine coronary arterial in 1979^[3]. One year later in 1980, Furchgott and Zawadzki et al.^[4] reported the relaxation of arterial smooth muscle by acetylcholine required the presence of endothelial cells and it is a substance named endothelium-derived relax factor (EDRF) released from the endothelial cells that caused the relaxation. Several years later, Ignarro et al.^[5,6] and Moncada et al.^[7,8] hypothesized and demonstrated that EDRF and NO is the same chemical molecule with pharmacological and chemical evidences on their own, respectively.

In fact, a producer of NO, nitroglycerin, has already been the powerful medicine for coronary heart diseases and angina pectoris for more than 100 years since it was first synthesized by the Italian chemist Ascanio Sobrero in 1847. Alfred Nobel, who was the instituter of the Nobel Prizes and invented dynamite of nitroglycerin refused to take nitroglycerin as a medicine for his coronary heart disease and therefore succumbed to the lingering heart ailment. Two months before his death in December, 1896, he wrote to a friend: " My heart trouble will keep me here in Paris for another few days at least, until my doctors are in complete agreements about my immediate treatment. Isn't it the irony of fate that I have been prescribed nitroglycerin, to be taken internally! They call it trinitrin, so as not to scare the chemist and the public."

Despite the mournful tragedy, nitroglycerin can truly re-

lieve the symptoms of angina pectoris through dilating the collateral vessels of the coronary arteries and correcting the imbalance between oxygen supply and demand of the heart. On the other hand, the underlying mechanism of relaxation of the smooth muscle cell by NO is the stimulation of soluble guanylate cyclase which subsequently convert to cyclic guanosine monophosphate (cGMP) and dephosphorylate the myosin light chain^[9]. The group of nitrovasodilators included nitroglycerin and sodium nitroprusside exert their effects through generating NO in the body. Robert Furchott, Ferid Murad and Louis Ignarro won the 1998 Nobel prize for discovering the role of NO as a signaling molecule in the cardiovascular system. The identity of NO turned out to be a new biological regulator, which could significantly reduce the morbidity of angiocardopathy. The significant numbers of publications and the Nobel prize related to NO did not hinder the progress related to NO studies and related translational implications. As a matter of fact, the innovative exploration on NO was advanced further as discussed below.

NO acts as a selective local pulmonary vasodilator

Warren Zapol, A physician scientist at Harvard Medical School didn't satisfy with the "breakthrough of zero". Based on existing evidences, he hypothesized that NO could be a selective pulmonary vasodilator to treat hypoxic pulmonary vasoconstriction. He successfully linked the following evidences together: Firstly, NO can dilate the bovine coronary arterial; Secondly, endogenous NO also can inhibit the hypoxic pulmonary vasoconstriction; And thirdly, the pharmacological effects of NO can be inactivated rapidly through binding hemoglobin with high affinity. He successfully demonstrated that "inhaled NO can act as a selective pulmonary vasodilator without causing systemic vasodilation" in lamb models of pulmonary hypertension^[10], indicating that NO could be a novel alternative for neonatal pulmonary hypertension treatment. However, such hypothesis was hardly to be accepted then. In 1990, at a symposium on the possible therapeutic usage of NO for childhood pulmonary disorders held at the NIH, one member of the scientific panelists mocked, tongue-in-cheek: "If truly NO is so beneficial, then why do not we set up pediatric hospital beds alongside the Los Angeles freeways?" Time will tell, and fortunately this time was not too late. In 1992, Roberts et al. reported on the *Lancet* that inhalation up to 80ppm NO rapidly and significantly increased pre- and post-ductal oxygen saturation (SpO₂) as well as oxygen tensions in infants with persistent pulmonary hypertension of the newborn (PPHN) and did not cause systemic hypotension or raise methemoglobin^[11]. The exciting results accelerated the popularization of inhaled NO with PPHN.

NO inhalation is a general accepted life-saving and effective therapy for pulmonary hypertension now. Currently

there are about 50,000 newborns benefit from NO therapy annually. In order to increase its availability and lower cost, the Zapol's group recently designed a NO generator from ambient air or oxygen-nitrogen mixtures^[12]. It will be safer and more convenient for the patients, especially in emergency situations.

NO exerts cardioprotective effect with UV exposure

Innovative thought is always just a flash in the brain whereas be hindered by the conventional mind set. For instance, the higher morbidity of angiocardopathy in winter is obviously, and easily to attribute it to vasoconstriction related with low temperature. Indeed there have been articles reported the promotion for the atherosclerotic plaque growth and instability by cold stimulation^[13].

However the researchers would stick to keep investigating the underlying mechanisms instead of stop at the surface. In England and Wales, the mortality appears to be correlated with latitude with the results analyzed by some researchers that it is 23% higher in the most northern (55° latitude) than the most southern (50°) and ischemic heart disease is one of the major reasons for the overall excess mortality^[14]. Similar to the intriguing phenomenon, the blood pressure (BP) is also correlated with latitude as it is lower in summer and near equator.

Hence, is sunlight good for our health? Professor Richard Weller from University of Edinburg tried to answer the question with hypothesizing that NO which stored in human skin as nitrite or nitrosothiols and cleaved by UV is very likely to be the essential role involved in the correlation of low temperature and angiocardopathy through exerting coronary vasodilator and antihypertensive effects in systemic circulation. Following his evidence^[15] published, some other researchers found UV could lower BP with increased level of nitrite and UVA exposure of human skin releases epidermis NO from storage forms. The beneficial cardiovascular effects of UV in sunlight exert through transforming the stored nitrosyl-haem species to NO in skin to circulation.

NO is involved in the adverse effects of storage erythrocytes

Recently growing number of literature demonstrated the increased incidence of complications associated with the transfusion of stored red blood cell (RBC). Blackstone et al. investigated the mortality after cardiac surgery and reported that the survival of patients receiving blood stored for more than 14 days is lower than those receiving blood stored for less than 14 days^[16]. Although another retrospective study displayed the RBC storage duration is not associated with the mortality in general surgery patients^[17], the correlation of the survival for at least cardiovascular surgery and blood

storage was confirmed in a meta-analysis^[18].

The underlying mechanism of the RBC storage lesion is attributed to the NO scavenging by cell-free plasma hemoglobin during the storage over time^[19]. Transfusion of syngeneic leukoreduced murine RBCs (SRBCs, stored for 2 weeks) or the supernatant from SRBCs into endothelial dysfunctioned db/db mice produces systemic hypertension and vasoconstriction, which is result from NO scavenging by the hemolysis and posttransfusion hemoglobinemia^[20]. However, systolic blood pressure did not change in healthy volunteers with sustained endothelial function when autologous packed erythrocytes stored for 40 days was transfused^[21].

In some further studies inhaled NO ameliorated the adverse effects of resuscitation with stored erythrocytes in the mice^[22] and lambs^[23] with hemorrhagic shock. In conclusion, the morbidity and complications associated with the storage erythrocytes may result from the NO scavenging caused by increased cell free oxyhemoglobin concentrations.

Innovation is not so difficult as it may seem. What you should do is to be the first one to think of, the first one to do and the best one of all, then change the track when some other can do the same. What do you want to be? Ordinary clinician, scientific clinician, or clinical scientist? It is up to you who define yourself.

Conflict Interests Disclosure

The authors have no conflicting interests to disclose.

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