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Nitric oxide and reactive oxygen species: a common mechanism of multiple comorbidities

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Running title: ROS-NO related comorbidities

Abstract

Common comorbid diseases such as obesity, diabetes mellitus and hypertension are main causes of death and disability around the globe. Available medications of such complex diseases are symptomatic, do not target the underlying cause and lack precision. A core reason for this medical knowledge gap is that these multifaceted disorders are often described by symptoms in certain organ and not by a molecular causal mechanism. Systems medicine, however, shows that these comorbidities are closely linked and clustered within the human disease network (also known as the diseasome). Therefore, such clusters likely share common causal pathophysiological mechanisms, and targeting these pathomechanisms would be superior to symptom-based therapeutics. These mechanisms are not a single molecular target, yet small signaling networks or modules of multiple targets. Thus, targeting multiple targets in a single module by mechanistically related drugs i.e., network pharmacology is superior to single target strategies. In this mini review, we discuss one example of causal signaling modules consisting of targets related to reactive oxygen species (ROS) and nitric oxide (NO) signaling.

Keywords: Obesity, Diabetes, Hypertension, Nitric oxide, ROS

Introduction

The high incidence of common comorbidities necessiates a quick action plan to improve the management of patients with such conditions (Ording & Sorensen, 2013). Around one quarter of the adult population have two or more chronic diseases, and more than 50% of aged patients have three or more chronic conditions (Barnett et al., 2012; Cynthia M Boyd, 2012). Those "problematic" patients with multiple disorders are often excluded from clinical studies (Mercer et al., 2009). Besides, most of the available medications only alleviate symptoms and lack precision, as expressed by high number needed to treat (NNT). This represents how many individuals that should receive a drug within a specific period to attain benefit for one individual. For example, angiotensin receptor blockers, that are used to treat hypertension, 409 patients need to be treated for 4 years to prevent one mortality due to cardiovascular event (Brugts et al., 2015).

Knowledge gap

A core knowledge gap contributing to these low therapeutic benefits is due to how we currently describe diseases. Undeniably, many multifaceted diseases are described by symptoms in particular organ, the phenotype, and not by their causal mechanisms. Common

disorders, such as obesity and diabetes, are just umbrella terms for diverse disease mechanisms that share a principal symptom, e.g., increased body weight or high blood glucose, but have dissimilar comorbid conditions. Such complex diseases, could not be caused by dysfunction of a single gene/ protein, yet by a dysfunctional signaling network/ module. These disease modules could be targeted by a combination of low-dose multiple mechanistically related drugs i.e. network pharmacology. This approach will guarantee a synergistic effect and lessen adverse drug reactions (Casas et al., 2019; Hopkins, 2008).

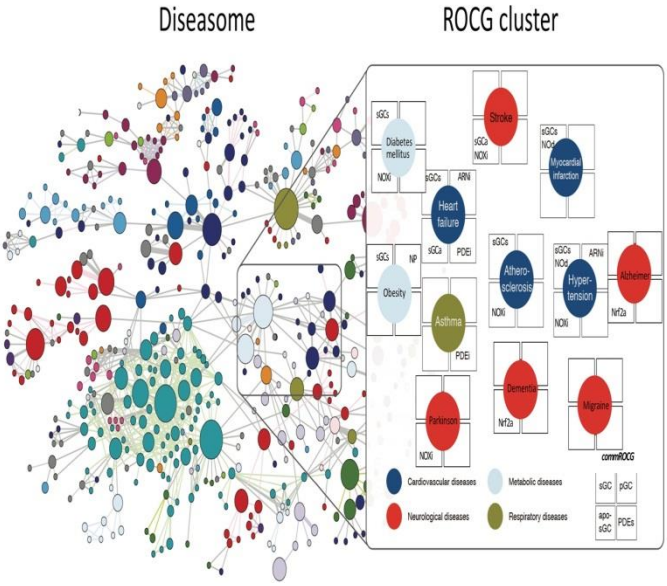

Figure 1. ***The ROCG cluster*** *(adapted from (Langhauser et al., 2018)). Abbreviations: ARNi, angiotensin receptor-neprilysin inhibitor; NOd, nitric oxide donors; NOXi, NADPH oxidase inhibitors; NP, natruretic peptides; Nrf2a, Nrf2 activators; PDEi, phosphodiesterase inhibitors; sGCa, soluble guanylate cyclase activators; sGCs, sGC stimulators.*

Reactive oxygen species-nitric oxide (NO) related comorbidities

The human disease network (the diseasome), in which diseases are connected by shared molecular mechanisms, was first described in 2007 (Goh et al., 2007). The diseasome shows that many diseases are closely associated and form clusters suggesting that they likely share same common pathomechanisms. Outstandingly, these clusters or common mechanisms could be used to describe diseases and allow drug repurposing/repositioning within disorders of the same cluster (Langhauser et al., 2018). One example of such disease clusters is one that includes twelve disease phenotypes from different organs e.g., hypertension, diabetes, heart failure, astham and Alzheimer's disease. These diseases were found to share a common causal network/module involving reactive oxygen species generation (ROS) and dysregulated NO-cyclic guanosine monophospahate (cGMP) (ROCG, Figure 1) (Langhauser et al., 2018).

This cluster was recently revalidated, based not only on genetic data, but also on protein-protein interactions (PPIs) (Langhauser et al., 2018). An important note here is that the above disease phenotypes may be caused by dysregulation of the ROCG network or by other modules. For instance, a patient X with hypertension and diabetes may have dysregulated ROCG, and patient Z with the same phenotypes may have other causative module. This means that many mechanisms can lead to the same phenotypes and each of these mechanisms just represents an endophenotype (subtype) that is most likely linked to dissimilar comorbid disorders.

Identification (diagnosis) of a ROCG patient might be via mechanism or module-based biomarkers. For instance, ROS (e.g., NADPH oxidase 5) and cGMP-related biomarkers (e.g., uncoupled NO synthase) have been utilised to identify ROCG patients in hypertension (Elbatreek et al., 2020).

Taken together, it is hypothesized that dysfunctionning of the ROCG module i.e.,

ROCG endophenotype/ mechanism, could be a disease description for some of the individuals with the above phenotypes. From a clinical perspective, if a disease is mechanistically well-described, one should be able to precisely diagnose and treat this disease. Thus, a comprehensive understanding and validation of the ROCG module is indispensable.

Molecular targets of the ROCG module

The ROCG module consists of two interrelated pathways, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)-induced ROS production and NO signaling. First, regarding ROS, such as superoxide and hydrogen peroxide (H_2O_2), these species are formed within the body and have multiple functions in diverse physiological processes and regulate cell signaling and function (Vara & Pula, 2014). Dysregulation of ROS is linked to many disorders including atherosclerosis, diabetes, hypertension, heart failure, asthma, stroke and myocardial infarction (Casas et al., 2019; Elbatreek et al., 2019; Gray et al., 2013; Henricks & Nijkamp, 2001; Lassegue & Griendling, 2004). Several ROS sources within the body exist e.g., mitochondria, xanthine oxidase, uncoupled NO synthase (uc-NOS) and NOX enzymes (A. I. Casas et al., 2015; Dao et al., 2019). The latter family produces ROS as its sole function, while other sources have other functions in addition to ROS formation (Ana I Casas et al., 2015; Dao et al., 2015; Dao et al., 2019).

Second, concerning NO signaling, NO is an ubiquitous molecule that plays important physiological roles such as dilatation of blood vessels, hindering leukocyte adhesion and impeding platelet aggregation (Davignon & Ganz, 2004; Puzserova & Bernatova, 2016). NO is formed by three NOS isoforms (NOS1-3) and it mainly acts by stimulation of soluble guanylate cyclase (sGC) and consequent cGMP formation. cGMP is a second messenger that activates cGMP-dependent protein kinase G (PKG) and is metabolized by phosphodiesterases (PDEs) (Monica et al., 2016; Pacher et al., 2007).

Under disease conditions, ROS interact with NO/cGMP signaling at distinct points (Figure 2). First, ROS oxidize tetrahydrobiopterin, a NOS cofactor, thus transforming NOS to uc-NOS which forms superoxide resulting in reduced NO bioavailability (Gielis et al., 2011). Second, superoxide can chemically react with NO resulting in the formation peroxynitrite, which provokes cell death (Pacher et al., 2007). Third, ROS can convert sGC to the NO-unresponsive sGC (apo-sGC) (Evgenov et al., 2006; Oettrich et al., 2016; Stasch et al., 2006).

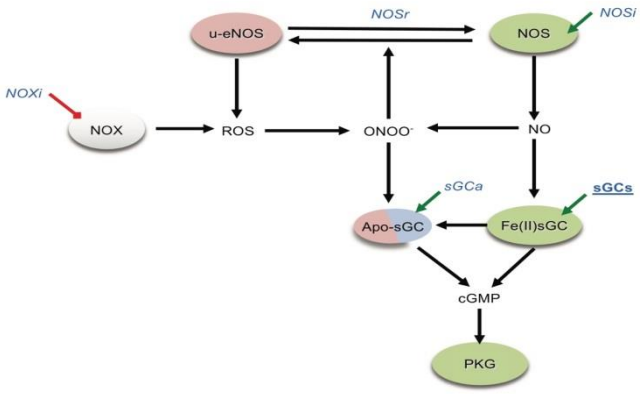

Figure 2. Molecular targets of the ROCG module. *NOX-produced ROS interfere with NO/ cGMP signalling via direct chemical reaction to form peroxynitrite ($ONOO^-$), uncoupling of NOS and oxidation of sGC to form apo-sGC. Multiple drugs can activate normal and inhibit abnormal signaling. Abbreviations: NOSi, NOS inhibitors; NOSr, NOS recoupling drugs; NOXi, NOX inhibitors; sGCa, sGC activators; sGCs, sGC stimulators. Abbreviations: cGMP, cyclic guanosine monophosphate; NO, nitric oxide; NOS, NO synthase; NOSi, NOS inhibitors; NOX, NADPH oxidase; NOXi, NOX inhibitors; PKG, protein kinase G; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; sGCa, sGC activators; sGCs, sGC stimulators; u-eNOS, uncoupled endothelial NOS. Adapted from (Oettrich et al., 2016).*

Potential therapeutic targets within the ROCG module

Diverse drugs can target the ROCG module in order to restore normal and efficient

cellular functions (Figure 2). NOX inhibitors e.g., setanaxib, display encouraging outcomes in experimental studies and are now tested in clinical trials of diabetic complications and lung fibrosis (Lee et al., 2020; Teixeira et al., 2017). NOS inhibitors show neuroprotective activities in animal models of ischemic stroke and are now examined clinically (Favie et al., 2018). NOS recoupling drugs which can revert uc-NOS to NOS, could be beneficial in long-lasting diseases with low NO levels such as diabetes and hypertension (Elbatreek et al., 2019; Gielis et al., 2011). sGC stimulators, recently approved for heart failure (vericiguat, NNT=24) and pulmonary hypertension (riociguat, NNT=6), stimulate sGC and are synergistic with little NO (Ghofrani et al., 2013). Finally, sGC activators e.g., cinaciguat, which are being tested in many clinical trials, can activate the NO-unresponsive apo-sGC (Sandner et al., 2019). Based on the underlying disease mechanism, these medications can be combined in low doses to restore normal signaling.

Conclusion

In conclusion, comorbidities are better described by shared causal mechanisms and not by symptoms or organs in order to precisely diagnose and treat these disorders. One such causal disease mechanism is dysregulated ROS and NO signaling (ROCG module). This network/module is associated with a group of comorbidities including obesity, diabetes, hypertension, heart failure and asthma, among others. Multiple molecular targets within the ROCG module can be targeted to restore normal physiological condition. These comprise, NOX enzymes (NOX4 and NOX5), NOS isoforms (NOS1 and NOS3) and sGC (both heme-containing sGC and heme-free, oxidised apo-sGC). Combining two or more of these drugs in low doses could have synergistic effect and reduce adverse effects.

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أكسيد النيتريك وشوارد الأكسجين التفاعلية: آلية مشتركة للعديد من الأمراض المصاحبة

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الأمراض المصاحبة الشائعة مثل السمنة والسكري وارتفاع ضغط الدم هي الأسباب الرئيسية للوفاة والعجز في جميع أنحاء العالم. العلاجات الحالية تستهدف الأعراض، ولا تستهدف السبب الأساسي لهذه الأمراض، وبالتالي فهي تعتبر علاجات غير دقيقة. أحد الأسباب الرئيسية لهذه الفجوة الطبية هو أن هذه الأمراض المعقدة يتم تعريفها أو تشخيصها من خلال عرض في أحد أعضاء الجسم وليس من خلال الآليات الجزيئية المسببة لها. ومع ذلك، يُظهر الطب الشبكي أنه داخل شبكة الأمراض (diseasome)، ترتبط هذه الأمراض المصاحبة ببعضها في مجموعة واحدة، وبالتالي، من المحتمل أن تشترك هذه الأمراض في نفس الآليات المسببة لها. وبالتالي يُفضل استهداف هذه الآليات المسببة بدلا من استهداف الأعراض، وبما أن هذه الآليات عبارة عن شبكة صغيرة من البروتينات، فإن علم الأدوية الشبكي الذي يقوم علي استهداف أكثر من بروتين يعتبر أفضل من الأساليب العلاجية التي تستهدف بروتين واحد فقط. في هذه البحث المصغر، نناقش مثالًا واحدًا من الآليات المسببة للأمراض المصاحبة وهو شوارد الأكسجين الحرة مسار إشارات أكسيد النيتريك.