Journal of Hypertension: Open Access

Porpino et al., J Hypertens 2016, 5:3
DOI: 10.4172/2167-1095.1000232

Review OMICS International

Developing New Organic Nitrates for Treating Hypertension: A Review

Suenia K. P. Porpino[1], Renata A. Travassos[1], Danilo D. A. Gadelha[1], Camille M. Balarini[1,2], Josiane C Cruz[1], Alexsandro F. Santos[3], Petrônio F. Athayde-Filho[3], Valdir A. Braga[1*] and Maria S. França-Silva[1]

[1]*Biotechnology Center, Federal University of Paraíba, João Pessoa, PB, Brazil*

[2]*Health Sciences Center, Federal University of Paraíba, João Pessoa, PB, Brazil*

[3]*Department of Chemistry, Federal University of Paraíba, João Pessoa, PB, Brazil*

[*]**Corresponding author**: Valdir de Andrade Braga, DVM PhD, Biotechnology Center, Federal University of Paraiba, P.O. Box 5009, 58.051-970, João Pessoa, PB, Brazil, Tel: (55) 83 32167173; Fax : (55) 83 32167173; E-mail: valdir@cbiotec.ufpb.br

Received date: October 21, 2015; **Accepted date:** September 21, 2016; **Published date:** September 28, 2016

Abstract

Nitric oxide (NO) is one of the most important vasodilator molecules produced by endothelium and presents plenty of cardiovascular actions. It has already been established that deficiency in NO/cGMP signaling pathway is involved in pathophysiological mechanisms of many cardiovascular diseases. In this context, the use of NO-releasing drugs appears to be an effective alternative to replace the deficient endogenous NO and mimic the role of this molecule in the body. Organic nitrates represent the oldest class of NO donors that have been clinically applied. Considering that tolerance can occur when these drugs are used chronically, the search for new compounds of this class with lower tolerance potential is increasing. Here we briefly discuss the mechanisms involved in nitrate tolerance and highlight some achievements from our group in the development of new organic nitrates and their preclinical application in cardiovascular disorders.

Keywords: Organics nitrates; Nitrate tolerance; 2-nitrate-1,3-dibuthoxypropan

Introduction

Nitric oxide (NO), first described as endothelium-derived relaxing factor (EDRF), is one of the most important mediators of intra- and extracellular processes. It is produced endogenously by the action of the enzyme NO synthase and exerts its effects mainly by activation of soluble guanylate cyclase (sGC), increasing the production of the intracellular second messenger cyclic GMP (cGMP) and activation of cGMP-dependent protein kinase (PKG) pathways [1]. Reduced bioavailability of NO is involved in the pathophysiology of many cardiovascular disorders. Therefore, the use of NO donor drugs, including organic nitrates, appears to be an effective alternative to replace the deficient endogenous NO and mimic the role of this molecule in the body [2].

Organic nitrates are nitric acid esters of mono- and polyhydric alcohols, representing the oldest class of NO donors that have been clinically applied. The main representatives of this class are nitroglycerine (NTG), isosorbide dinitrate (ISDN), isosorbide 5-mononitrate (ISMN) and pentaerythritol tetranitrate (PETN) [3]. All these compounds present a similar molecular structure, with the nitrate ester bond (R-O-NO_2) as an essential feature. This chemical group confers unique biological properties to this class of compounds, based on NO release [4,5].

Nitric oxide donors and, more specifically, organic nitrates have been used for many years in the treatment of cardiovascular disorders such as angina pectoris, arterial hypertension, heart failure, preeclampsia and pulmonary hypertension [6-9]. Despite the benefits exercised by organic nitrates to the cardiovascular system, the prolonged use of these drugs may cause tolerance [10]. Here we discuss some mechanisms involved in nitrate tolerance and present further information about new insights on this class of drugs, especially in new molecules developed and tested in preclinical trials by our group.

Nitrate tolerance

The development of nitrate tolerance is characterized by the reduction of its vasodilator effect and the requirement to use continuouslyhigher doses, consisting in the major limiting factor to clinical use of these drugs [11].

The first report of nitrate tolerance was in 1905, when Stewart [12] noted that prolonged use of NTG led to reduction of the side effects of redness and headache. Several animal studies have confirmed the development of nitrate tolerance in all species tested, both *in vivo* and *in vitro* [13-16]. This phenomenon may have several causes and remain incompletely clarified, despite numerous studies in search of clarifications.

Tolerance appears to result from phenomena at the systemic level, such as neurohormonal activation and intravascular volume expansion or vascular changes like inhibition of nitrate biotransformation, desensitization of the soluble guanylyl cyclase (sGC) and cGMP-dependent protein kinase (PKG), increase in phosphodiesterase activity and uncoupling of the NO synthase [17].

Evidence indicates that oxidative stress is closely related in all processes of tolerance to organic nitrates, such as NTG, ISDN, and ISMN. This hypothesis is supported by numerous reports demonstrating that tolerance is prevented or, at least, ameliorated by co-administration of antioxidants such as vitamin C, vitamin E and folic acid. Moreover, interventions inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent formation of reactive oxygen species (ROS) with statins, angiotensin converting enzyme (ACE) inhibitors, hydralazine and apocynin exhibit also

promising effects against tolerance [10,18-21]. However, the exact mechanisms involved in this phenomenon remain unclear.

New insights

Due to tolerance caused by organic nitrates, the search for new compounds of this class which are unable to induce this undesirable effect has been increasing in the last years. Recently, our research group evaluated new organic nitrates obtained from glycerin, which is generated as a byproduct of the biodiesel production [22,23]. Four compounds were obtained.

Frist, we evaluated if these compounds were able to induce vascular relaxation, the main feature of organic nitrates. It was observed that all the tested organic nitrates caused vasorelaxation in superior mesenteric artery rings (SMAR) isolated from rats. The nitrates-induced relaxation was concentration-dependent and endothelium-independent. Table 1 shows the percentage of maximal relaxation (maximum effect – ME) induced by these compounds and the potency of each one (as pD_2, the negative logarithm of the concentration that produces half of the maximum effect). Data previously published in França-Silva et al. [23].

Compounds	Intact endothelium		Denuded endothelium	
	(%) ME ± SEM	pD_2 ± SEM	(%) ME ± SEM	pD_2 ± SEM
NDMP	88.5 ± 11.2	4.7 ± 0.13	93.8 ± 11.7	4.4 ± 0.07
NDEP	94.1 ± 6.7	4.6 ± 0.08	108.8 ± 5.4	4.8 ± 0.06
NDPP	96.4 ± 8.3	5.5 ± 0.10	111.1 ± 8.5s	5.4 ± 0.08
NDBP	89.5 ± 3.4	5.8 ± 0.10	105.4 ± 2.7	5.9 ± 0.06

Table 1: Maximum Effect (ME) and pD_2 values of organic nitrates derived from glycerin. The relaxation was evaluated in superior mesenteric artery isolated from rats precontracted with phenyleprine (10 μM), in the presence or absence of functional endothelium as described in França-Silva et al. [24] (n = 6 for each group). The vasorelaxant effect was expressed as a percentage of relaxation of phenylephrine-induced contraction and data were expressed as mean ± S.E.M.

Although no significant differences were found in ME and pD_2 values between the components studied, we observed a tendency towards increasing the vasodilator response as the number of carbon atoms in the carbonic chain increased. Thus, we chose NDBP (2-nitrate-1,3-dibuthoxypropan) to further test its mechanism of action. Our laboratory had shown that NDBP evoked potent vasorelaxation through NO generation and activation of the sCG/cGMP/PKG pathway. The kinetic of NO release was similar to other NO donors such as glyceryl trinitrate (NTG) and sodium nitroprusside (SNP) [25,28], confirming this molecule as a new organic nitrate that acts as a NO donor. Apparently, NDBP releases NO both by non-enzymatic and enzymatic pathways. Enzymatic pathways depend, at least in part, of xanthine oxidase activity, once its inhibition reduces NDBP-mediated NO production [28]. In agreement with these findings, *in vivo* preclinical studies proved that administration of different doses of NDBP (1, 5, 10, 15 and 20 mg/kg) elicited dose-dependent hypotension in spontaneously hypertensive rats (SHR) [26]. In addition to its NO-donor properties, our recent data also suggests that NDBP decreases oxidative stress by reducing NADPH oxidase activity both *in vivo* and *in vitro* [28].

Considering that tolerance is one of the most important undesirable effect evoked by organic nitrates, we performed experimental trials to evaluate the ability of NDBP to induce tolerance. Experimental protocols were conducted according to Daiber et al. [27]. The cumulative addition of NDBP (10-8 to 10-4 M) in SMAR isolated from another group of rats promoted vasodilation with ME = 111 ± 4% and pD_2 = 5,8 ± 0.06. Previous exposition of SMAR to NDBP (10 μM or 100 μM) for 30 minutes did not alter the vasorelaxant response induced by cumulative addition of the compound (ME = 113 ± 1%, pD_2 = 6.0 ± 0.04; ME = 111 ± 6%; pD_2 = 5.8 ± 0.07, respectively, suggesting that NDBP did not induce tolerance to vasodilatation in vitro. In a different subset of experiments, SMAR were exposed to NDBP during 60 minutes and this did not change de relaxation to cumulative doses of the compound [28], suggesting NDBP does not cause tolerance like other organic nitrates such as nitroglycerin. One limitation of the study with NDBP was the fact that cross-tolerance with NTG was not tested. Also, we still need to perform experiments to confirm *in vivo* tolerance-induction and the classical toxicology tests in different rodent and other mammals. This will allow further phase I clinical tests.

These data confirm the promising role of NDBP as a NO donor with potential to be used clinically on cardiovascular system. Preclinical studies confirmed that this molecule has anti-hypertensive effects due to its ability to release NO and decrease oxidative stress. However, further studies regardingits potential to induce tolerance need to be performed, as well as additional studies with the others organic nitrates. These compounds can help our understanding on nitrate tolerance mechanisms and possibly can figure as new potential therapeutic alternatives for cardiovascular disorders in substitution to current available NO donors.

References

1. Förstermann U, Sessa WC (2012) Nitric oxide synthases: regulation and function. Eur Heart J 33: 829-837.
2. Pörsti I, Paakkari I (1995) Nitric oxide-based possibilities for pharmacotherapy. Ann Med 27: 407-420.
3. Wang PG, Xian M, Tang X, Wu X, Wen Z, et al. (2002) Nitric oxide donors: chemical activities and biological applications. Chem Rev 102: 1091-1134.
4. Al-Sa'Doni H, Ferro A (2000) S-Nitrosothiols: a class of nitric oxide-donor drugs. Clinical Science 98: 507-520.
5. Miller MR, Megson, IL (2007) Recent developments in nitric oxide donor drugs. British Journal of Pharmacology 151: 305-321.
6. Miller MR, Wadsworth RM (2009) Understanding organic nitrates – A vein hope? British Journal of Pharmacology 157: 565-567.

7. Kalidindi M, Velauthar L, Khan K, Aquilina J (2012) The role of nitrates in the prevention of preeclampsia: an update. Curr Opin Obstet Gynecol 24: 361-367.
8. Maul H, Longo M, Saade GR, Garfield RE (2003) Nitric oxide and its role during pregnancy: from ovulation to delivery. Curr Pharm Des 9: 359-380.
9. Follmann M, Griebenow N, Hahn MG, Hartung I, Mais FJ, et al. (2013) The chemistry and biology of soluble guanylate cyclase stimulators and activators. Angew Chem Int Ed Engl 52: 9442-9462.
10. Münzel T, Daiber A, Mülsch A (2005) Explaining the phenomenon of nitrate tolerance. Circ Res 97: 618-628.
11. Klemenska E, Beręsewicz A (2009) Bioactivation of organic nitrates and the mechanism of nitrate tolerance. Cardiology Journal 16: 11-19.
12. Stewart DD (1905) Remarkable tolerance to nitroglycerin, Philadelphia Polyclinic, August, From Stewart, DD, Tolerance to nitroglycerin. JAMA 44: 1678-1679.
13. Bennett BM, Schroder H, Hayward LD, Waldman SA, Murad F (1988) Effect of in vitro organic nitrate tolerance on relaxation, cyclic GMP accumulation, and guanylate cyclase activation by glyceryl trinitrate and the enantiomers of isoidide dinitrate. Circ. Res 63: 693-701.
14. Münzel T, Sayegh H, Freeman BA, Tarpey MM, Harisson GD (1995) Evidence for enhanced vascular superoxide anion production in nitrate tolerance. J Clin Invest 95: 187-194.
15. Heitzer T, Just H, Brockhoff C, Meinertz T, Olschewski M, et al. (1998) Long-term nitroglycerin treatment is associated with supersensitivity to vasoconstrictors in men with stable coronary artery disease: prevention by concomitant treatment with captopril. J Am Coll Cardiol 31: 83-88.
16. Parker JD, Farrell B, Fenton T, Cohanim M, Parker JO (1991) Counterregulatory responses to continuous and intermittent therapy with nitroglycerin. Circulation 84: 2336-2345.
17. Münzel T, Daiber A, Gori T (2011) Nitrate Therapy: New Aspects Concerning Molecular Action and Tolerance. Circulation 123: 2132-2144.
18. Daiber A, Mulsch A, Hink U, Mollnau H, Warnholtz A, et al. (2005) The oxidative stress concept of nitrate tolerance and the antioxidant properties of hydralazine. Am J Cardiol 96: 25-36.
19. Mayer B, Beretta M (2008) The enigma of nitroglycerin bioactivation and nitrate tolerance: News, views and troubles. Br J Pharmacol 155: 170-184.
20. Daiber A, Wenzel P, Oelze M, Munzel T (2008) New insights into bioactivation of organic nitrates, nitrate tolerance and cross-tolerance. Clin Res Cardiol 97: 12-20.
21. Wenzel P, Mollnau H, Oelze M, Schulz E, Wickramanayake JM, et al. (2008) First evidence for a crosstalk between mitochondrial and NADPH oxidase-derived reactive oxygen species in nitroglycerin-triggered vascular dysfunction. Antioxid Redox Signal 10: 1435-1447.
22. Santos AF (2009) Novas Perspectivas da Glicerina – Síntese de Novos Nitratos com Propriedades Farmacológicas e Melhoradores de Cetano. Dissertação (Mestrado) - Universidade Federal da Paraíba, João Pessoa.
23. França-Silva MS, Balarini CM, Cruz JC, Khan BA, et al. (2014) Organic Nitrates: Past, Present and Future. Molecules 19: 15314-15323.
24. Kirk-Othmer ET (2007) Glycerol, in: American Society of Chemistry. Encyclopedia of chemical technology, New York, John Wiley.
25. França-Silva MS, Monteiro MMO, Queiroz TM, Santos AF, Athayde-Filho PF, et al. (2012) The 2-nitrate-1,3-dibuthoxypropan, a new nitric oxide donor, induces vasorelaxation in mesenteric arteries of the rat. European Journal of Pharmacology 690: 170-175.
26. França-Silva MS, Monteiro MMO, Queiroz TM, Santos AF, Athayde-Filho PF, et al. (2012). The new nitric oxide donor 2-nitrate-1,3-dibuthoxypropan alters autonomic function in spontaneously hypertensive rats. Autonomic Neuroscience: Basic and Clinical 171: 28-35.
27. Daiber A, Oelze M, Coldewey M, Bachschmid M, Wenzel P, et al. (2004) Oxidative stress and mitochondrial aldehyde dehydrogenase activity: a comparison of pentaerythritol tetranitrate with other organic nitrates. Molecular Pharmacology 66: 1372-1382.
28. Porpino SK, Zollbrecht C, Peleli M, Montenegro MF, Brandão MC, et al. (2016) Nitric oxide generation by the organic nitrate NDBP attenuates oxidative stress and angiotensin II-mediated hypertension. Br J Pharmacol 173: 2290-2302.