Role of nitric oxide in the pathogenesis of dengue

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Abstract

The free radical nitric oxide (NO) has emerged in recent years as a fundamental signalling molecule for the maintenance of homeostasis, as well as a potent cytotoxic effector involved in the pathogenesis of a wide range of human diseases. The presence of NO during dengue infection as well as its experimental antiviral and apoptotic effects have been documented. In this regard, increased serum NO levels in dengue fever and basal levels in the haemorrhagic form of dengue have been reported. Clinical and experimental data suggest that NO could act as a beneficial factor during dengue infection by its antiviral and apoptotic effects; however, more intense investigations are required.

Keywords: Dengue virus; Nitric oxide; Dengue haemorrhagic fever, Pathogenesis.

Introduction

Dengue fever (DF) is the most important arboviral disease affecting public health in the developing world. The disease is endemic in many countries and causes epidemics frequently. These epidemics involve an estimated 100 million cases annually worldwide, causing great health, social and economic burden.[1,2] Dengue virus (DENV), a RNA virus belonging to genus, Flavivirus, family Flaviridae, has four serotypes (DENV-1 to 4). Clinically, the disease may be asymptomatic or may range from a mild febrile illness, DF, to the severe, life-threatening form, dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), which can be caused indistinctly by any serotype.[3-5]

The discovery that mammalian cells have the ability to synthesize the free radical nitric oxide (NO) has stimulated an extraordinary impetus for scientific research in all fields of biology and medicine. NO is produced by three isoforms of nitric oxide synthase (NOS), the inducible form (iNOS), and the two constitutive isoforms endothelial NOS (eNOS) and neuronal NOS (nNOS). Since its early description as an endothelial-derived relaxing factor, NO has emerged as a fundamental signalling device regulating virtually every critical cellular function, as well as a potent mediator of cellular damage in a wide range of conditions. Recent evidence indicates that most of the cytotoxicity attributed to NO is rather due to peroxynitrite, produced from the diffusion-controlled reaction between NO and another
free radical, the superoxide anion. Peroxynitrite interacts with lipids, DNA, and proteins via direct oxidative reactions or via indirect, radical-mediated mechanisms. These reactions trigger cellular responses ranging from subtle modulations of cell signalling to overwhelming oxidative injury, committing cells to necrosis or apoptosis. In vivo, peroxynitrite generation represents a crucial pathogenic mechanism in conditions such as stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, neurodegenerative disorders and viral infections.⁶⁻⁹

Previous reports have suggested a possible role of NO in DF and DHF/DSS pathogenesis,¹⁰⁻¹⁷ however, very little information is available with regard to its role during the human dengue infection. Therefore, evidence from in vivo and in vitro conditions suggesting the possible role of NO in dengue infection is presented in this review.

**Nitric oxide in dengue infection**

NO is ubiquitous physiological-free radical that is responsible for many pathological disorders.⁶⁻⁹ Its presence in human dengue infection was reported for the first time by Valero and collaborators.¹⁰

In this regard, increased serum NO levels in DF and normal levels in DHF patients were reported. There was no relationship between the viral serotypes and the increased values of NO. Interaction of dengue virus with primary human platelet cultures did not induce further regulation of NO production.¹⁰ These data suggest that increased levels of NO in DF patients could have a protector effect during the disease, and, on the contrary, decreased levels could be the cause of the deleterious effects observed in DHF. In addition, it seems to be that virus-platelets interaction is not involved in the increased production of NO, suggesting other sources for this molecule. In this regard, monocyte/macrophages are the most important targets of DENV,¹⁸ and are capable of producing high amounts of NO; thus, Mo/MΦ-DENV interaction may induce increased amount of NO. In accordance with that suggestion, increased expression of inducible nitric oxide synthase in monocytes from DF patients has been reported,¹³ which translates into sustained NO production,¹³,¹⁵,¹⁶ suggesting the possible source of NO found in patients with DF.

Since endothelium is an important NO producer, the direct or indirect interaction of DENV with the endothelial cells could induce an increased amount of serum NO in these patients.⁶⁻⁸,¹⁶,¹⁷ However, increased levels of NO in patients with dengue seem to be controversial. Other investigators¹⁹ have reported levels of serum NO significantly lower than those of normal controls in Asiatic children with DF and DHF, suggesting that the endothelial damage renders the endothelium incapable of producing NO. These data are different from the increased amount of serum NO found in patients with DF reported by others.¹⁰ This different response to the virus infection could be related to the widespread prevalence of human dengue resistance genes in the Americas’ populations compared to Asian populations. The people of countries in South-East Asia have high rates of severe dengue disease compared to consistently low case-fatality rates in American countries reporting DHF.²⁰ Virtually, nothing is known about dengue resistance gene(s) in the pathogenesis of dengue. Are the manifestations of DF suppressed by this gene(s), or does the gene(s) selectively dampen dengue vasculopathy?²⁰ The expression of this gene(s) could involve different cell targets during the disease leading
to differential production of NO. Future research on dengue infections should emphasize population-based designs to shed light on the heterogeneity of dengue in populations living in the Western and Eastern hemispheres.

**Anti-viral effect of nitric oxide**

The beneficial effect of NO in DF could be related to an antiviral effect of the molecule. Previous studies have shown that NO could have antiviral effect on dengue-infected cells. In this regard, decreased expression of dengue virus antigens in NO-producing mononuclear cells has been reported. In addition, treatment of those cultures with an iNOS inhibitor (N\(^\circ\)-methyl-L-Arginine) induced increased viral antigens detection.\(^{13}\) The effect of NO exogenous donor (S-nitroso-N-acethylpenicillamine or SNAP) on DENV replication in neuroblastoma cell cultures has also been reported. DENV-2-infected cells treated with SNAP showed suppression of viral RNA synthesis and, consequently, decreased viral proteins and viral progeny production.\(^{12}\) This effect was also observed in DENV-1-infected C6/36 cell cultures treated with sodium nitroprussiate, a NO donor.\(^{13}\) Takhampunyna et al.\(^{14}\) showed the inhibitory effect of NO on DENV-infected LLC-MK2 cell cultures using SNAP. This report found a diminished cellular accumulation of viral RNA during viral RNA synthesis. This inhibitory effect of NO seems to be related to the inhibition of DENV-2 RNA-dependent RNA polymerase (RdRp) domain of NS5, with further suppression of viral RNA synthesis, viral structural proteins, and decreased number of infectious particles in the culture medium.

Recently, Ubol et al.\(^{21}\) reported DENV-2 clinical isolates derived from DF and DHF cases, which are NO-sensitive and NO-resistant, respectively, to endogenous NO produced by THP-1 cells (human monocytic leukemia cell line). These findings were also related with amino acids changes in the RdRp and Methyltransferase (MTase) domains of NS5. Additionally, when compared the gene expression on DENV-2 infected THP-1 cells, NO-resistant but no NO-sensitive isolates, induced the immune-related genes IL-6, IL-7, IL-8, RANTES, and MCP-3, all of which are potent mediators of tissue damage as well as coagulopathy. These findings need to be carefully studied to determine if this effect is particularly restricted to DENV-2 or to its Asian genotype, or if NO-resistant strains occurs in the nature for the other serotypes.

All the previous data suggest a possible antiviral mechanism of NO in vivo, and suggest a beneficial role of increased serum levels of NO reported in DF.\(^{10}\) Lower levels of NO found in patients with DHF could be related to the described DENV action on target cells. In this regard, Chareonsirisuthigul et al.\(^{11}\) showed a different antiviral response in experiments using DENV-infected THP-1 cell cultures untreated and treated with an anti-DENV-enhancing antibodies (a model of infection via ADE). These experiments showed suppression of NO production in ADE cultures due to disruption of transcription of the IRF-1 (an iNOS gene transcription factor) and blocking of the activation of STAT-1. These findings could be related to the pathogenesis of DHF/DSS and could suggest a mechanism for basal serum NO levels in DHF patients.\(^{10}\)

This antiviral effect of NO is not restricted to the dengue virus. Several studies have reported a NO antiviral effect on an extensive list of viruses including SARS-coronavirus, hantavirus, Ross River virus, vesicular stomatitis virus, Crimean-Congo haemorrhagic fever virus, and members of the genus *Flavivirus*, including Japanese encephalitis.\(^{22-29}\)
Nitric oxide and apoptosis

The dengue virus may be an apoptosis inducer by direct or indirect mechanisms. Infection of Mo/MΦ or endothelial cells with DENV results in apoptosis,[30-32] however, the mechanisms of apoptosis induction remain unclear. The free radical NO has emerged in recent years as a fundamental signalling molecule for the maintenance of homeostasis, as well as a potent cytotoxic effector. Under normal conditions, NO produced in low concentration acts as a messenger and cytoprotective (antioxidant) factor; however, when the circumstances allow the formation of substantial amounts of NO, this molecule could induce both oxidative and nitrosative stresses, which form the basis of the apoptosis generally attributed to NO.[7-9]

Previous investigations have shown the presence of apoptosis in patients both with dengue and in experimental dengue infection.[30-34] However, there is little information about the role of NO in apoptosis during dengue infection. In this regard, antibodies against dengue virus nonstructural protein 1 (NS1), which has cross-reactivity with endothelial cells (AECA), induce apoptosis via a NO-mediated mechanism in experimental dengue virus infection. Endothelial cells undergo apoptosis via the mitochondria-dependent pathway that is regulated by NO production, suggesting that NO-regulated endothelial cell injury may play a role in the disruption of vessel endothelium and contribute to the pathogenesis of vasculopathy.[16,17] Other investigators have shown increased apoptosis in dengue virus-replicating Kupffer cells associated with increased expression of inducible NO synthase and production of NO.[15] Apoptosis could avoid the release of viral particles and, together with the phagocytosis and digestion of apoptotic cells, represent mechanisms to prevent viral progeny.[32] However, damage of endothelial cells and monocytes/macrophages could induce severe forms of dengue infection. Further investigation is required to determine the modulation of NO in order to provide the therapeutic strategies for dengue infection by preventing the AECA-mediated endothelial cell apoptosis and to determine the in vivo mechanism of NO-mediated apoptosis induced by DENV during the course of human dengue infection.

Conclusion and future perspective

NO, on the basis of its physiological chemistry, provides a conceptual framework, which helps to distinguish between the beneficial and toxic consequences of NO, and to envision potential therapeutic strategies for the future. The high levels of NO may be beneficial during dengue infection by its antiviral and apoptotic effects; however it could also induce severe forms of the disease by damage to endothelial cells. Further investigations are required to determine the source of NO during human dengue infection, its role in the pathogenesis of DF and DHF/DSS, its role in dengue infection according to the prevalence of human dengue resistance genes, and the presence of NO-resistant strains in all the DENV serotypes.

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