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Micro-anatomical changes in major blood vessel caused by dengue virus (serotype 2) infection

Sivan Padma Priya[a], S. Sakinah[a], Mok Pooi Ling[b,c], Hui-Yee Chee[a], Akon Higuchi[d,e,f], Rukman Awang Hamat[a], Vasantha Kumari Neela[a], Abdullah A. Alarfaj[f], Murugan A. Munusamy[f], Ashraf A. Hatamleh[f], Ahmed E. Al-Sabri[f], Ibrahim Ahmad Abdulaziz Al-Suwailem[f], Mariappan Rajan[g], Giovanni Benelli[h], Marlina[i], S. Suresh Kumar[a,c,j,*]

[a] Department of Medical Microbiology and Parasitology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[b] Department of Biomedical Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[c] Genetics and Regenerative Medicine Research Centre, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[d] Department of Chemical and Materials Engineering, National Central University, Jhong-li, Taoyuan, 32001 Taiwan
[e] Department of Reproduction, National Research Institute for Child Health and Development, Tokyo, 157-8535, Japan
[f] Department of Botany and Microbiology, King Saud University, Riyadh, 11451, Saudi Arabia
[g] Biomaterials in Medicinal Chemistry Laboratory, Department of Natural Products Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai-21, Tamil Nadu, India
[h] Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy
[i] Faculty of Pharmacy, Universitas Andalas, Padang, West Sumatera, Indonesia
[j] Innoscience Research, Kuala Lumpur, Malaysia

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ABSTRACT

Dengue virus (DENV) has emerged as a major economic concern in developing countries, with 2.5 billion people believed to be at risk. Vascular endothelial cells (ECs) lining the circulatory system from heart to end vessels perform crucial functions in the human body, by aiding gas exchange in lungs, gaseous, nutritional and its waste exchange in all tissues, including the blood brain barrier, filtration of fluid in the glomeruli, neutrophil recruitment, hormone trafficking, as well as maintenance of blood vessel tone and hemostasis. These functions can be deregulated during DENV infection. In this study, BALB/c mice infected with DENV serotype 2 were analyzed histologically for changes in major blood vessels in response to DENV infection. In the uninfected mouse model, blood vessels showed normal architecture with intact endothelial monolayer, tunica media, and tunica adventitia. In the infected mouse model, DENV distorted the endothelium lining and disturbed the smooth muscle, elastic laminae and their supporting tissues causing vascular structural disarrangement. This may explain the severe pathological illness in DENV-infected individuals. The overall DENV-induced damages on the endothelial and it's supporting tissues and the dysregulated immune reactions initiated by the host were discussed.

1. Introduction

Dengue virus, from the genus *Flavivirus*, includes four serotypes and causes dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). The virus is mainly transmitted through the *Aedes aegypti* mosquito bites and has emerged as a major economic concern in developing countries, with 2.5 billion people believed to be at risk, globally (Halstead 2007; Clark et al., 2005). Dengue infection may cause the individual to experience more severe illness with subsequent infections, which may increase the chances of developing DHF and DSS (Arevalo et al., 2009; Halstead, 2003; Noisakran and Perng, 2008; Rothman, 2010). Clinical signs of DHF/DSS occur due to increased capillary permeability of endothelium, including altered hemostasis, thrombocytopenia, as well as rapid and widespread plasma leakage in surrounding tissues and cavities of the body (Halstead, 2002, 2007; Chaturvedi and Shrivastava, 2004).

Previously, the endothelium was thought to function only as a membrane that is selectively permeable to electrolytes, gas and water. The detailed discussion about the various functions of the endothelium reported (Rajendran et al., 2013). The vascular endothelial cells (ECs)

* Corresponding author at: Department of Medical Microbiology and Parasitology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
E-mail address: sureshkudsc@gmail.com (S.S. Kumar).

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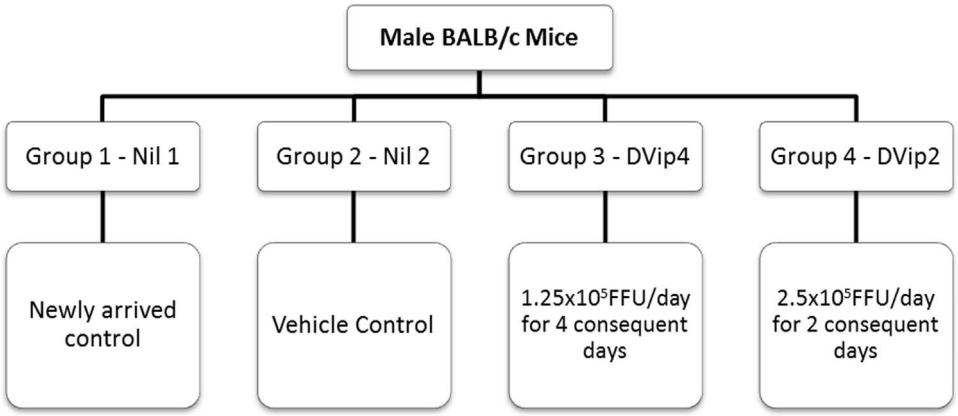

Fig. 1. Schematic diagram of the study plan. Nil 1 represents uninfected mice, sacrificed after 1 week; Nil 2 represents uninfected mice that were administered 100 µl of phosphate-buffered saline, sacrificed at day 21; DVip4 represents mice intraperitoneally administered dengue virus for 4 consequent days; DVip2 represents mice intraperitoneally administered dengue virus for 2 consequent days.

not only line the circulatory system, but also perform distinct functions including gas exchange in lungs, gaseous, nutritional and its waste exchange in all tissues involving the blood brain barrier, fluid filtration in the glomeruli, neutrophil recruitment, hormone trafficking, as well as maintenance of blood vessel tone and hemostasis (Durand and Gutterman, 2013). In addition, ECs do regulate blood flow by controlling platelet and leukocyte interactions with the vessel wall, thrombosis, thrombolysis, regulation of vascularity, vasculogenesis and augmentation of blood vessels (Verhamme and Hoylaerts, 2006).

Endothelial functions are disrupted in response to infection by dengue virus. Dengue viruses can infect diverse type of cells including peripheral leukocytes, liver cells, endothelial cells and dendritic cells, as revealed by previous reports of patients who died due to dengue (Diamond et al., 2000; Jessie et al., 2004; Oishi et al., 2007) and in animal models (Balsitis et al., 2009; Balsitis et al., 2010; Chen et al., 2007) and *in vitro* (Ubol et al., 2010).

However, investigating the effect of dengue virus infection on endothelial cells in living patients is a challenge. Additionally, most pathology studied to date involved organs such as the liver, brain, and kidney, whereas reports on the structures of major blood vessel are limited(Sakinah et al., 2017). Minor blood vessels like end arteries and capillaries (microcirculation), are prone for leakage to any minor insult due to their natural arrangement. EC damages in microcirculations directly exposes the underlying thin basement membrane (BM), which can be easily breached by the viral and host immune reactions leading to leakage of inflammatory fluid and blood cells. The extend of leakage is proportional to the level of damage in EC and BM. However, in respect to major blood vessels like aorta and pulmonary veins and arteries, the endothelial layer named tunica intima (Ti) damage exposes a thick muscular and elastic layer named tunica media (Tm), which is again covered by a third layer named tunica adventitia (Ta) of loose connective tissue (Junqueira and Mescher, 2010) that proposes different manifestations to dengue infections. The multilayered structural presentation prevents the hemorrhage or leakage in major blood vessels (which is the hallmark of dengue in minor blood vessels), nevertheless the underlying layers are disturbed by direct and indirect impact of the DENV2.

Vascular smooth muscle cells (VSMC) are important for blood volume distribution and they maintain it by controlled contraction and relaxation with the help of special intercellular gap junctions with Ti endothelial cells (Durand and Gutterman, 2013). They are unique in their function and needs to be investigated because, life-threatening diseases like aortic dissection are due to deregulated functions of SMC of Tm. Even though by phenotypic classification, the smooth muscle cells (SMC) are distributed from small blood vessels to uterus, they do differ in specific gene expressions and functional modifications in respect to different locations. The morphological and histological variations of vascular SMC (VSMC) between thoracic aorta and abdominal aorta (were noted, which are continuous structures of same locations) explains the high diversity in SMC. The studies on VSMC, elastic laminae and the fibroblasts of Ta in aspect to dengue infection were reported less. Therefore, in the present study, we investigated the effect of dengue (serotype 2) infection on the histology of endothelial lining and their supporting tissues in the major blood vessel of BALB/c mice.

2. Materials and methods

2.1. Virus

Dengue virus type 2 (DENV-2; strain 3738) used in this study was maintained in confluent C6/36 cells (ATCC CRL-1660) for 8 days at 28 °C under 5% CO_2. The supernatant was collected in 15-ml tubes and stored at −80 °C. The virus stock used in this study was passaged 10 times in cell cultures to obtain an adequate amount of virus stock. Foci forming assay was performed to quantify the DENV- 2 stock and the titer obtained was 5×10^5 foci-forming units (FFU)/ml (Mota and Rico-Hesse 2011; Martinez-Gutierrez et al., 2014; Goncalves et al., 2012).

2.2. Animals

Male BALB/c mice, weighing 25 g to 30 g were obtained from Sinar Scientific, Malaysia. The mice were fed pallet diet and housed under ordinary conditions in the animal house at the Faculty of Medicine and Health Sciences. All protocols involving animals were approved by the animal ethics committee of IACUC, Universiti Putra Malaysia, ref. no. UPM/IACUC/AUP-R017/2015.

2.3. Infection by DENV-2

After 1 week of acclimatization, mice were randomly divided into four groups, which are Group 1 (newly arrived control), Group 2 (vehicle control), Group 3 (4 continuous day infected with dengue virus) and Group 4 (2 continuous day infected with dengue virus) (Fig. 1). Group 3 and 4 were intraperitoneally administered with 5×10^5 FFU DENV-2 for four (1.25×10^5 FFU/day) and two consecutive days (2.5×10^5 FFU/day), respectively (Martinez-Gutierrez et al., 2014).