Journal of Infection (2015) 71, 480—487

BIAM
British Infection Association

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Decreased plasma levels of the endothelial protective sphingosine-1-phosphate are associated with dengue-induced plasma leakage

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Accepted 22 June 2015
Available online 14 July 2015

KEYWORDS
Sphingosine 1-phosphate;
Dengue;
APOM protein;
Human;
Blood platelets

Summary *Background:* A transient endothelial hyperpermeability is a hallmark of severe dengue infections. Sphingosine-1-phosphate (S1P) maintains vascular integrity and protects against plasma leakage. We related plasma S1P levels to dengue-induced plasma leakage and studied mechanisms that may underlie the decrease in S1P levels in dengue.
Methods: We determined circulating levels of S1P in 44 Indonesian adults with acute dengue and related levels to plasma leakage, as determined by daily ultrasonography, and to levels of its chaperone apolipoprotein M, other lipoproteins and platelets.
Results: Plasma S1P levels were decreased during dengue and patients with plasma leakage had lower median levels compared to those without (638 vs. 745 nM; $p < 0.01$). ApoM and other lipoprotein levels were also decreased during dengue, but did not correlate to S1P levels. Platelet counts correlated positively with S1P levels, but S1P levels were not higher in frozen-thawed platelet rich plasma, arguing against platelets as an important cellular source of S1P in dengue.
Conclusions: Decreased plasma S1P levels during dengue are associated with plasma leakage. We speculate that decreased levels of ApoM underlies the lower S1P levels. Modulation of S1P

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http://dx.doi.org/10.1016/j.jinf.2015.06.014

levels and its receptors may be a novel therapeutic intervention to prevent plasma leakage in dengue.

Introduction

Dengue is a systemic mosquito-borne viral infection for which no vaccine or specific treatment exists. This disease is endemic to tropical and subtropical areas of the world causing a fundamental public health problem.[1] Clinical manifestations vary from a mild self-limiting febrile illness to a severe life-threatening condition. Complications of dengue usually arise around the time of defervescence and include bleeding manifestations, thrombocytopenia and plasma leakage.[2] The latter is due to a transient endothelial hyperpermeability, of which the pathogenesis is still incompletely understood.[3]

Several studies implicate that the bioactive sphingolipid sphingosine 1-phosphate (S1P) is a crucial signaling molecule that modulates a variety of physiological functions. Some of the pleiotropic actions of S1P are highly relevant for dengue infection, including the regulation of cell death and the modulation of immune cell trafficking, but especially the maintenance of the endothelial integrity.[4,5] The multiplicity of S1P-mediated actions can be explained by the fact that the sphingolipid on the one hand exhibits intracellular targets and on the other hand is able to stimulate G protein-coupled receptors (GPCR) after release into the extracellular environment. Until now five high-affinity receptors for S1P, designated S1P1—S1P5, have been discovered. S1P1 has been identified as the central receptor subtype involved in the robust barrier enhancing action of S1P.[6]

In mammalians high levels of S1P are mainly present in the circulatory system such as blood and lymph and its cellular source has only recently begun to be characterized. Erythrocytes contain large amounts of S1P[7,8] and are together with vascular endothelial cells[9] a main source for blood S1P. Additionally, platelets are an important storage site for S1P[10] which can be released upon platelet activation.[11,12] In the circulation, S1P is largely chaperoned by apolipoprotein M (ApoM), which is a plasma apolipoprotein that associates with different lipoproteins, but particularly with high-density lipoproteins (HDL).[13] Hepatocytes can generate and release ApoM-lipoprotein and its overexpression is associated with increased plasma S1P concentrations[14] and larger ApoM-S1P enriched HDL particles in mice.[15] ApoM concentrations correlate to lipoprotein concentrations, above all to total cholesterol levels.[16] A reduction in plasma cholesterol concentrations is commonly observed during dengue and is related to severity of illness and mortality.[17]

Recently, Gomes et al. reported decreased S1P serum levels during dengue infection with the lowest levels in those with severe dengue infection.[18] To investigate the role of reduced S1P levels in plasma leakage in dengue as well as mechanisms involved in the reported low S1P levels in dengue, we measured plasma S1P levels in a cohort of adult Indonesian dengue patients and related these levels to plasma leakage, as determined by daily ultrasonography, serum lipoprotein and ApoM levels as well as platelet counts.

Methods

Patients and study design

This prospective observational study was part of a larger study in which we included patients aged 14 years and above who were admitted to Rumah Sakit Hasan Sadikin, an academic referral hospital in Bandung, West Java, Indonesia, from March 2011 to March 2012.[19] Patients with a history of fever and thrombocytopenia ($<150 \times 10^9$ cells/L) and with clinical suspicion of dengue infection were included. Exclusion criteria were presence of a concurrent chronic disease, pregnancy, the inability to retrospectively obtain a laboratory confirmation of dengue diagnosis. Patients from whom no platelet rich plasma (PRP) was obtained were also excluded from the current study. Demographic, clinical, laboratory and ultrasonography data were collected using a standardized data collection form. Ultrasonography was daily performed at the bedside to detect plasma leakage as a complication of dengue as described in detail before.[20] Routine complete blood count was determined every day. Additionally, blood was drawn at enrollment of the study and in each clinical phase of dengue infection; the febrile phase (temperature of 37.5 °C or higher); the critical phase (period within 48 h of defervescence when complications usually occur and before platelet count recover); the early recovery phase (increasing platelet counts with a minimum increase of $10*10^9$/L, which further increases in the days thereafter until normal platelet counts are reached) and the convalescence phase (>2 weeks after discharge). Plasma leakage was defined as an increase in hematocrit of ≥20%, a single high hematocrit value (>50% for men and >44% for women), and/or by plasma leakage in the form of ascites and/or pleural effusion detected by ultrasonography, according to WHO guidelines.[21] Plasma leakage within the gallbladder wall is an early sign of plasma leakage and can be observed by ultrasonography as a thickened gallbladder wall, but it is not part of the WHO criteria for plasma leakage.[21] Gallbladder wall thickness (GBWT) was therefore not used as a criteria for plasma leakage in this study. Circulatory failure was defined as a narrow pulse pressure (a difference between systolic and diastolic blood pressure of 20 mmHg or less), and/or hypotension (decrease in systolic blood pressure of 20 mmHg or more compared to the normal blood pressure, if this known for a specific patient), and/or a systolic blood pressure below 100 mmHg in combination with clinical signs of hypoperfusion. All patients were asked to return for follow up in the convalescent phase 2—4 weeks after discharge, which