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Mediators of vascular leak in dengue infections

Malavige G N[1]

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Abstract

Although plasma leakage is the hallmark of severe dengue infections the factors that cause increased vascular permeability have not been identified. As platelet activating factor (PAF), lipid mediators and cytokines are associated with increase in vascular permeability, we set out to investigate the role of these mediators in acute dengue infection.

Levels of inflammatory lipid mediators and cytokines were initially evaluated in 25 patients with confirmed acute dengue infection and 12 healthy individuals. After determining which inflammatory mediators were associated with severe dengue, serial assays of the above lipid mediators, and cytokines were done in an additional 36 patients in serum samples collected 12 hours apart throughout the course of their hospital stay.

We found that PAF levels were significantly higher in patients (p=0.001) when compared to healthy individuals and that PAF levels rose just before the onset of the critical phase. PAF values of >100ng/ml were associated with reduced expression of gap junction proteins leading to increased vascular permeability. A similar effect was seen with serum from patients with dengue infection which was reversed by a PAF receptor antagonist. S1P levels which are associated with increasing the integrity of the endothelial barrier were significantly reduced in patients with DHF. Serum IL-10, TNFα and IL-1β levels were elevated in patients with DHF and followed the same patterns as PAF. In conclusion, our results show that PAF is likely to be a potent mediator of vascular leak in dengue and use of PAF blockers could be therapeutically useful.

Introduction

Dengue viral infection has become one of the most important mosquito borne viral infections in the world and is one of the major emerging infectious diseases. It has been predicted that 390 million dengue infections occur per year resulting in approximately 96 million clinically apparent infections.[1] As a result of the high disease burden due to dengue infections, it has been declared a priority infection by the WHO, UNICEF and World Bank.[2]

Sri Lanka has had several dengue epidemics for the past 21 years, and the incidence and severity of these epidemics is increasing.[3] The incidence of dengue has been particularly high since 2009 (Figure 1) and the scarce resources available in most hospitals in Sri Lanka have been stretched to the limits. Early detection of shock and other complications and supportive therapy has shown to reduce the morbidity and mortality.[4,6] The case fatality rates which were 0.99% in year 2009 and 0.74% in year 2010[7] has been halved in more recent years due to the enormous efforts taken by physicians, paediatricians and other health care personnel.

Currently there are no effective antiviral drugs to treat acute infection, nor an effective vaccine to prevent infection. However, with early detection of complications and proper fluid management, case fatality rates can be kept very low. Although Sri Lanka and many other countries have achieved this with tremendous effort, it is a significant burden on resource poor hospitals such

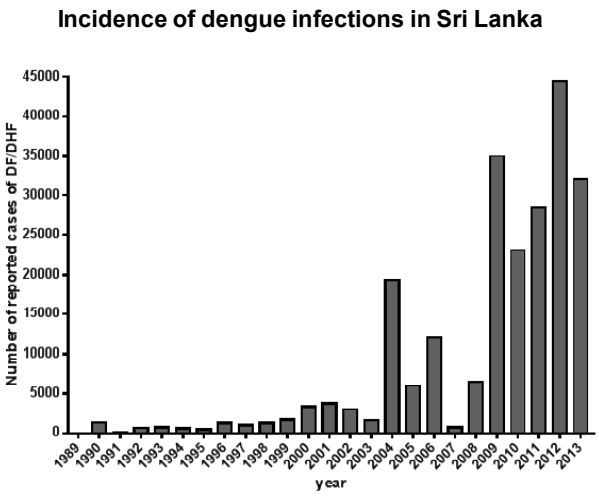

Figure 1. Incidence of dengue infections in Sri Lanka from 1989 to 2013. Source: Sri Lanka Epidemiology unit

[1] *Director, Centre for Dengue Research, Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka.*
E-mail: neelikamalavige@gmail.com

as ours. Therefore, early identification of patients who are likely to develop severe disease and complications would enable clinicians to act quickly in order to minimize severe disease.

Vascular leak in dengue infection

The dengue virus (DV) potentially infects many cell types[8], and disseminates around the body in the viraemic phase (febrile phase) which may last for 2-7 more days[2]. The patient may then progress to the critical phase which is characterized by plasma leakage.[4] Complications as a result of plasma leakage such as shock, pleural effusions, ascites along with other complications such as liver failure and encephalopathy may occur during the critical phase.[2] The critical phase usually lasts for 24-48 hours before the patient enters the recovery phase. Some patients however, enter the recovery phase without progressing through the critical phase.

Many factors are thought to play a role in causing endothelial dysfunction in dengue infections. Cytokines and other mediators produced by monocytes have shown to act on the endothelium and cause increased vascular permeability.[9,11] In addition, *in vitro* studies have shown that mediators released from mast cells also cause endothelial activation and increased vascular permeability, especially in the presence of dengue immune sera[12,13] Although many mediators have been implicated as possible causes of endothelial dysfunction and vascular leakage, these studies have been based on *in vitro* data.

Mediators that cause vascular leak

Many mediators such as platelet activating factor (PAF), tumour necrosis factor alpha (TNFα), vascular endothelial growth factor (VEGF), sphingosine 1 phosphate (S1P), monocyte chemo attractant protein -1 (MCP-1) and thrombin have been shown to cause increased vascular permeability in sepsis and anaphylactic shock.[14,17] The factors that cause vascular leak are thought to be present in serum of dengue patients, as sera from dengue patients have shown to reduce expression of gap junction proteins thus leading to vascular leak.[18] Among the potential mediators that cause vascular leak, VEGF has been extensively studied in dengue,[19,20] and it has been shown that free plasma VEGF levels are significantly higher and VEGF receptor 2 levels are significantly lower in those with DHF.[20] In addition, the rise in VEGF and fall in VEGFR2 was shown to correlate with the onset of vascular leak.[20] Apart from VEGF, many studies have also shown that TNFα and MCP-1 were increased in DHF, [21,22] although some studies including studies done by us have shown contradictory results.[23,24]

Platelet activating factor (PAF) as a mediator of vascular leak

In our most recent studies we evaluated the role of PAF and other lipid mediators in the pathogenesis of vascular leak. PAF is a phospholipid mediator with many biological functions including increasing vascular permeability.[25] It is rapidly synthesized from many cells such as endothelial cells, leucocytes and monocytes in response to cellular stress.[25,26] Intravenous administration of PAF in animal experiments was shown to result in hypotension, thrombocytopenia and increased vascular permeability.[27,29] PAF has also been shown to be associated with vascular leak in mice models of dengue infection, which was reversed by PAF inhibitors called UK-74,505.[30] In addition, PAFR knockout mice were shown to be less susceptible to developing severe dengue.[30] Since, PAF was shown to be associated with vascular leak in mice models, we proceeded to determine its role in human dengue infections. We found that PAF levels were significantly elevated in patients with DHF when compared to those with DF, throughout the course of the illness. Since we found that PAF breakdown enzyme levels were also similarly higher in patients with DHF when compared to DF, the rise of PAF is likely to be due to increased production.[31]

As PAF levels were higher in patients with DHF and tend to rise just before the onset of the critical phase associated with fluid leakage, we also investigated if PAF indeed caused increased vascular permeability. These experiments were carried out in Human Umbilical Vein Endothelial cell lines (HIVECs) using a PAF agonist, PAF antagonist and serum from patients with dengue infection. The effect of these mediators on HUVECs was determined by assessing the expression of a tight junction protein, ZO-1 by confocal microscopy.[32] We found that sera from patients with dengue infection resulted in a significant reduction in ZO-1 expression, which was upregulated when the HUVECs were pre-treated with a PAF antagonist. Therefore, these experiments conclusively showed that PAF indeed was a cause of vascular leak in acute dengue.[31]

Sphingosine 1 – phosphate (S1P) as a mediator of vascular leak

Sphingosines are a family of lipids and when phosphorylated by sphingosine kinases 1 and 2 generate S1P.[33,34] S1P has been shown to protect endothelial barrier integrity induced by VEGF.[34] S1P antagonists have shown to disrupt endothelial barrier integrity in a dose dependent manner.[34] In addition, S1P is shown to oppose the inflammatory effects of PAF and reduce vascular leakage induced by PAF in rat models.[35] In summary, S1P appears to have a

protective role against vascular leak and maintains endothelial barrier integrity. Therefore, we proceeded to investigate the role of S1P in acute dengue infection. We found that S1P levels were significantly lower in patients with severe forms of dengue. S1P was lowest in those who developed grade IV DHF.[36] S1P levels particularly reduced during the critical phase in patients with DHF suggesting that a reduction in S1P during the critical phase, is also likely to contribute to increased vascular permeability.[36]

Summary

Our recent research of the mediators of vascular leak shows that PAF levels were significantly associated with vascular leak. In contrast, S1P which is important in endothelial cell integrity, was detected at lower levels in patients with DHF when compared to DF, especially during the critical phase. Drugs that block PAF receptors and those that potentiate the action of S1P are already available and are used for other disease conditions. Therefore, the use of these drugs in the treatment of dengue and their effect on reducing vascular leak should be evaluated.

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