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PERSONAL INFORMATION

Age, Date of Birth	34 years, 30th August 1984
Gender	Male
Marital status	Married
Blood group	B –ve.
Languages known	English, Malayalam, Hindi, Tamil, Thai
Nationality	Indian
Passport details	No. Z3141196, Place of Issue: Cochin; DOI: 17-04-2015, DOE: 16-04-2025.

OBJECTIVE:

To obtain a research cum teaching position which allows me to develop as an academician, thereby contributing my experience and enthusiasm to a healthy, growing and learning community.

ACADEMIC QUALIFICATIONS:

DOCTOR OF PHILOSOPHY (Ph.D.); Biochemistry (Medical Program); August 2013.
Mahidol University, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand.
Thesis: Role of Extracellular-Signal Regulated Kinase (ERK) in a Mouse Model of Dengue Virus Mediated Liver Injury (**Thesis Advisor**: Dr. Thawornchai Limjindaporn, MD, Ph.D.)

MASTER OF PHILOSOPHY (M.Phil.); Biochemistry; December 2009.
Vinayaka Mission University, Directorate of Distance Education, Salem, Tamil Nadu, India.

POST GRADUATE DIPLOMA (PG. Diploma)**;** Hospital Management; May 2008.
Annamalai University, Directorate of Distance Education, Annamalai Nagar, Tamil Nadu, India.

MASTER OF SCIENCE (M.Sc.); Biochemistry; March 2007.
Annamalai University, Faculty of Science, Annamalai Nagar, Tamil Nadu, India.

BACHELOR OF SCIENCE (B.Sc.); Biochemistry (Chemistry and Zoology); March 2005.
University of Kerala, Thiruvananthapuram, Kerala, India (NSS College, Pandalam).

HIGHER SECONDARY EDUCATION (HSE); Science Group; March 2002.
Board of Higher Secondary Education, Thiruvananthapuram, Kerala, India (NSHSS, Mannar).

SECONDARY EDUCATION - Secondary School Leaving Certificate (SSLC); March 2000.
Board of Secondary Education, Thiruvananthapuram, Kerala, India.

PROFESSIONAL EXPERIENCE:

Research Associate

Mahidol University, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand.

Siriraj Center of Research Excellence for Molecular Medicine, Faculty of Medicine Siriraj Hospital, Department of Research and Development Mahidol University, Bangkok Noi, Bangkok, Thailand-10700.

20th August 2013 onwards.

- Host-viral interactions in dengue virus (DENV) infection
- Animal models of DENV infection
- Molecular therapeutics to DENV infection (drug repurposing, molecular inhibitors and natural compounds)
- DENV-mediated Mitogen Activated Protein Kinases (MAPK) signaling

Research Co-ordinator

Amrita Vishwa Vidyapeetham University, Amrita Institute of Medical Sciences & Research Center, Kochi, Kerala, India-682041.

Department of Dermatology & Cosmetology

16/09/2008 to 12/04/2010.

- Co-ordinator for the Research & Development (R&D) and Clinical trials
- Assistant for the cell culture procedure with the Melanocyte Transplantation Unit.

Project Fellow

Amrita Vishwa Vidyapeetham University, Amrita Institute of Medical Sciences & Research Centre, Kochi, Kerala, India-682041.

Department of Surgical Oncology/OGCA

28/06/2007 to 21/01/2008.

Project: "Prostate Cancer Screening in Kerala" as part of an Early Detection Clinic

Principal Investigator: Dr. K Chitrathara, MD, MCH, FAIS - Funded by **Kerala State Council for Science, Technology and Environment,** Thiruvananthapuram, Kerala, India.

PUBLICATIONS:

[1] **G.P. Sreekanth**, J. Panaampon, A. Suttitheptumrong, A. Chuncharunee, J. Bootkunha, P.T. Yenchitsomanus, T. Limjindaporn, **2019**. Drug repurposing of N-acetyl cysteine as antiviral against dengue virus infection. ***Antiviral research*** (Accepted). **Impact Factor-4.307**

[2] A. Chuncharunee, S. Waikakul, A. Wongkajornsilp, V. Chongkolwatana, L. Chuncharunee, A. Sirimontaporn, T. Rungruang, **G.P. Sreekanth***, Invalid freeze-dried platelet gel promotes wound healing, ***Saudi Pharm J***, 27 (**2019**) 33-40. **(Corresponding author). Impact Factor-3.110**

[3] K.K. Ajeeshkumar, K.V. Vishnu, R. Navaneethan, K. Raj, K.R. Remyakumari, T.R. Swaminathan, M. Suseela, K.K. Asha, **G.P. Sreekanth***, Proteoglycans isolated from the bramble shark cartilage show potential anti-osteoarthritic properties, ***Inflammopharmacology (Springer Nature)***, (**2019**). **(Corresponding author). Impact Factor-3.304**

[4] **G.P. Sreekanth**, P.T. Yenchitsomanus, T. Limjindaporn, Role of mitogen-activated protein kinase signaling in the pathogenesis of dengue virus infection, ***Cellular signalling***, 48 (**2018**) 64-68. **Impact Factor-3.487**

[5] A. Morchang, R.C.H. Lee, P.T. Yenchitsomanus, **G.P. Sreekanth**, S. Noisakran, J.J.H. Chu, T. Limjindaporn, RNAi screen reveals a role of SPHK2 in dengue virus-mediated apoptosis in hepatic cell lines, ***PloS one***, 12 (**2017**) e0188121. **Impact Factor-2.766**

[6] **G.P. Sreekanth**, A. Chuncharunee, B. Cheunsuchon, S. Noisakran, P.T. Yenchitsomanus, T. Limjindaporn, JNK1/2 inhibitor reduces dengue virus-induced liver injury, ***Antiviral research***, 141 (**2017**) 7-18. **Impact Factor-4.307**

[7] S.L. Leela, C. Srisawat, **G.P. Sreekanth**, S. Noisakran, P.T. Yenchitsomanus, T. Limjindaporn, Drug repurposing of minocycline against dengue virus infection, ***Biochemical and biophysical research communications***, 478 (**2016**) 410-416. **Impact Factor-2.559**

[8] **G.P. Sreekanth**, A. Chuncharunee, A. Sirimontaporn, J. Panaampon, S. Noisakran, P.T. Yenchitsomanus, T. Limjindaporn, SB203580 Modulates p38 MAPK Signaling and Dengue Virus-Induced Liver Injury by Reducing MAPKAPK2, HSP27, and ATF2 Phosphorylation, ***PloS one***, 11 (**2016**) e0149486. **Impact Factor-2.766**

[9] **G.P. Sreekanth**, A. Chuncharunee, A. Sirimontaporn, J. Panaampon, C. Srisawat, A. Morchang, S. Malakar, P. Thuwajit, S. Kooptiwut, A. Suttitheptumrong, P. Songprakhon, S. Noisakran, P.T. Yenchitsomanus, T. Limjindaporn, Role of ERK1/2 signaling in dengue virus-induced liver injury, ***Virus research***, 188 (**2014**) 15-26. **Impact Factor-2.484**

[10] F. Kaliyadan, J. Manoj, A.D. Dharmaratnam, **G. Sreekanth**, Self-learning digital modules in dermatology: a pilot study, ***Journal of the European Academy of Dermatology and Venereology***: JEADV, 24 (**2010**) 655-660. **Impact Factor-4.287**

[11] F. Kaliyadan, **S. Gopinathan Pillai**, The use of Google language tools as an interpretation aid in cross-cultural doctor-patient interaction: a pilot study, (**2010**), ***Journal of Innovation in Health Informatics***. **Impact Factor-0.480**

[12] F. Kaliyadan, M. Bhaskaran, A.D. Dharmaratnam, J. Manoj, **G. Sreekanth**, Anti-phospholipid syndrome preceding a diagnosis of lepromatous leprosy, ***Dermatology online journal***, 15 (**2009**) 4. **Impact Factor-0.910**

[13] F. Kaliyadan, A.D. Dharmaratnam, M.G. Jayasree, **G. Sreekanth**, Linear verrucous hemangioma, ***Dermatology online journal***, 15 (**2009**) 15. **Impact Factor-0.910**

CONFERENCE PUBLICATIONS / CONFERENCE PROCEEDINGS:

[1] **Sreekanth Gopinathan Pillai**, Aporn Chuncharunee, Pa-Thai Yenchitsomanus, Thawornchai Limjindaporn, **17th International Congress of Virology (IUMS-2017 Singapore) - Role of activating transcription factor-2 in dengue virus-induced liver injury (Poster Presentation)**

[2] **Sreekanth Gopinathan Pillai**, Aporn Chuncharunee, Aunchalee Sirimtaporn, Chatchawan Srisawat, Pa-thai Yenchitsomanus, Thawornchai Limjindaporn - ICMPH 2013, Faculty of Medicine Siriraj Hospital (Mahidol University), Bangkok, Thailand **"Role of MAPK's inhibitors in DENV mediated organ injuries in a mouse model"** - **Siriraj Medical Journal**, June 2013 **(Conference Publication and Oral Presentation)**

[3] **Sreekanth Gopinathan Pillai**, Aporn Chuncharunee, Juttatip Panaampon, Pa-thai Yenchitsomanus, Thawornchai Limjindaporn – **International Congress on Medical Virology (ICMV2014)**, The Virology Association (Thailand), "SB203580, an inhibitor of p38 MAPK, modulates the Dengue virus-mediated liver injury" – 5-7 November 2014, Bangkok, Thailand **(Conference Proceedings and Oral Presentation)**

[4] Shilu Malakar, Juttatip Panaampon, **Sreekanth G Pillai**, Pa-thai Yenchitsomanus, Thawornchai Limjindaporn – SIMPH 2014, Faculty of Medicine Siriraj Hospital (Mahidol University), Bangkok, Thailand. **"FR180204, A Selective Inhibitor of Extracellular Signal Regulated-Kinase Impedes Dengue Virus Replication *In Vitro"*** - **Siriraj Medical Journal**, Volume 66, Number 4, July-August 2014 **(Conference Proceedings and Oral Presentation)**

RESEARCH GRANTS:

[1] **Principal Investigator** - Grant No. R016120002; **Grant value:** 15,72,000 Thai Bhat;
Granting agency: Mahidol University, Thailand; **Duration:** Oct 2017 - Nov 2019.

[2] **Co-Investigator** - Grant No. R015810002); **Granting agency:** Mahidol University, Thailand.

[3] **Co-Investigator**-Siriraj Research and Development Grant No. R015533001,
Granting agency: Faculty of Medicine Siriraj Hospital; Mahidol University, Thailand.

[4] **Co-Investigator**-Siriraj Research and Development Grant No. R016134005,
Granting agency: Faculty of Medicine Siriraj Hospital; Mahidol University, Thailand.

HONORS & AWARDS

- **Invited Speaker** - **International Conference on Current Trends in Biosciences** (CTBio-2017)
  Scire Interactome Forum, Kerala, India.
- **Session Chair** - **International Conference on Current Trends in Biosciences** (CTBio-2017)
  Scire Interactome Forum, Kerala, India.
- **Travel Award** to attend **17th International Congress of Virology** (IUMS-2017), Singapore
  Singapore Society for Microbiology and Biotechnology (SSMB), Singapore.
- **Best Thesis Publication Award in Health Sciences**, Year 2013.
  Faculty of Graduate Studies, Mahidol University, Thailand.
- **Ph.D. Scholarship -** The Graduate Scholarship for International Students (Full Scholarship)
  Faculty of Medicine Siriraj Hospital (Mahidol University), Bangkok, Thailand.
- **Certificate of Appreciation -** Successful completion of Phase-II clinical study in Psoriasis.
  Biocon India, Bangalore, Karnataka, India.

PROFESSIONAL TRAINING

- Biosafety: Risk Assessment and SOP Development, Mahidol University, Thailand.
- Laboratory Animal Care - National Laboratory Animal Centre, Mahidol University, Thailand.
- Good Laboratory Practice (GLP) and Biohazards in good laboratory practices, Thailand.
- Bio-safety for animal studies (BSL-2), Mahidol University, Thailand.
- ICH-GCP, Amrita Vishwa Vidyapeetham University, Cochin, Kerala, India.

MEMBERSHIPS

- The American Society of Microbiology (ASM)
- Association of Clinical Biochemists in India (ACBI)
- European Association of Cancer Research (EACR)
- Society of Biological Chemist, India (SBCI, India)

CONFERENCE PARTICIPATIONS

- **Graduate Research Forum-2017 (GRF-2017);** 29 May 2017
  Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.
- **International Conference in Medicine and Public Health 2014 (ICMPH2014) -** July 21-25, 2014
  Ministry of Public Health of Thailand and Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand
- **International Conference in Medicine and Public Health 2013 (ICMPH2013) -** June 24-28, 2013
  Ministry of Public Health of Thailand and Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand
- **International Conference in Medicine and Public Health 2012 (ICMPH2012)**, Sep, 17-21, 2012.
  Ministry of Public Health of Thailand and Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand
- **ASM Virtual Workshop on Scientific Writing and Publishing**; July 13th, 2012
  The American Society of Microbiology-Thailand Chapter
- **International Conference on Thalassemia**; March 22-24, 2012.
  Thalassemia Center of Thailand and Mahidol University (Garden Cliff Resort & Spa, Pattaya, Thailand)
- **International Conference on Advances in Free Radical Research, Natural Products, Antioxidants and radio protectors in Health**; January 11-13, 2010
  Davis Heart & Lung Research Institute, Ohio State University & SFRR, NIMS, India (Hyderabad, India).
- **Indo-US CITI Workshop on Promoting Research Ethics Education in India**; November 24-25, 2009
  Sri Ramachandra University, Chennai, India & CITI, University of Miami, USA
- **ACBICON-2009** (Association of Clinical Biochemist of India Conference 2009; November 3-7, 2009
  Amrita Institute of Medical Sciences & Research Center, Kochi, Kerala, India.
- **CME on Current Topics in Microbiology**; August 8th, 2009
  Association of Clinical Microbiologist, India.
- **Workshop on Good Clinical Practices - Study conduct, Ethics, and Informed Consent**; Mar, 8, 2009
  Pfizer Ltd, USA and Amrita Institute of Medical Sciences (AIMS) Research collaboration
- **Investigators meeting for a multicenter clinical study**
  Actavis Mid- Atlantic, USA and Lotus Labs Pvt. Ltd, Bangalore, India.
- **National Seminar on Nutrigenomics: An innovative solution to lifestyle disorders** (UGC, India)
  Department of Industrial Microbiology, Government College for Women, University of Kerala, Thiruvananthapuram, Kerala, India.

PROFESSIONAL REFERENCES:

[1] Dr. Thawornchai Limjindaporn MD, Ph.D., Associate Professor & Deputy Dean of Post-Graduate Education Division, Department of Anatomy, Division of Molecular Medicine (Lab), Mahidol University Faculty of Medicine Siriraj Hospital, Wanglang Road, Bangkok Noi, Bangkok, THAILAND-10700. Phone: +66-863871506, Email: thawornchai.lim@mahidol.ac.th

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Research paper

Drug repurposing of N-acetyl cysteine as antiviral against dengue virus infection

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ABSTRACT

Liver injury is one of the hallmark features of severe dengue virus (DENV) infection since DENV can replicate in the liver and induce hepatocytes to undergo apoptosis. N-acetyl cysteine (NAC), which is a clinically-used drug for treating acetaminophen toxicity, was found to benefit patients with DENV-induced liver injury; however, its mechanism of action remains unclear. Accordingly, our aim was to repurpose NAC in the preclinical studies to investigate its mechanism of action. Time of addition experiments in HepG2 cells elucidated effectiveness of NAC to reduce infectious virion at pre-, during- and post infection. In DENV-infected mice, NAC improved DENV-associated clinical manifestations, including leucopenia and thrombocytopenia, and reduced liver injury and hepatocyte apoptosis. Interestingly, we discovered that NAC significantly reduced DENV production in HepG2 cells and in liver of DENV-infected mice by induction of antiviral responses via interferon signaling. NAC treatment in DENV-infected mice helped to maintain antioxidant enzymes and redox balance in the liver. Therefore, NAC reduces DENV production and oxidative damage to ameliorate DENV-induced liver injury. Taken together, these findings suggest the novel therapeutic potential of NAC in DENV-induced liver injury and recommend evaluating its efficacy and safety in humans with DENV-induced liver injury.

1. Introduction

Dengue virus (DENV) infection is one of several arboviral diseases that affect humans that are accelerating their spread into and across the tropical and subtropical regions of the world (Murray et al., 2013). Patients with DENV infection can develop any one of a range of disease severities, including self-controlling comparatively mild dengue fever (DF), dengue hemorrhagic fever (DHF), or the most severe dengue shock syndrome (DHS). Although all 4 of the DENV serotypes (serotypes 1–4) are-known to cause disease, the severity of disease was reported to differ among serotypes and strains (Fox et al., 2011; Vicente et al., 2016). The disease pathogenesis of DENV infection is complex with serotype specificity (Lin et al., 2011; Martina, 2014), and is still being investigated.

Liver injury was evident in DENV-infected patients (Samanta and Sharma, 2015), and was reported to be one of the major disease criteria for the severe forms of DENV infection by the World Health Organization (WHO) (Jayaratne et al., 2012). The clinical pathology of liver injury has been studied in DENV infection, and virus replication was found to be one of the factors that contributes most to liver impairment (Franca et al., 2010). In an immunocompetent mouse model of DENV infection exhibiting liver injury, inhibitors of mitogen-activated protein kinases (MAPKs) limited hepatic cell apoptosis and reduced liver injury in DENV-infected mice; however, these inhibitors were not able to restrict virus replication in the liver (Sreekanth et al., 2014, 2016, 2017, 2018).

Drug repurposing in the advancement of dengue antiviral development was recently reviewed (Low et al., 2018). In the present study, N-acetyl cysteine (NAC), a United States Food and Drug Administration (US FDA)-approved mucolytic drug and dietary supplement, is widely used as an antioxidant (Mokhtari et al., 2017), and to treat acetaminophen (also known as paracetamol) overdose (Yoon et al., 2016) was repurposed against DENV-induced liver injury. Two cohort studies

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Review

Role of mitogen-activated protein kinase signaling in the pathogenesis of dengue virus infection

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ABSTRACT

Dengue virus (DENV) infection is a disease that is endemic to many parts of the world, and its increasing prevalence ranks it among the diseases considered to be a significant threat to public health. The clinical manifestations of DENV infection range from mild dengue fever (DF) to more severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Increased proinflammatory cytokines and vascular permeability, both of which cause organ injury, are the hallmarks of severe dengue disease. Signs of liver injury were observed in studies using hepatic cell lines, mouse models, and autopsy specimens from DENV-infected patients, and these signs substantiated the effects of inflammatory responses and hepatic cell apoptosis. Mitogen-activated protein kinases (MAPK) are involved in inflammatory responses and cellular stress during viral infections. The roles of MAPK signaling in DENV infection were reviewed, and published data indicate MAPK signaling to be involved in inflammatory responses and hepatic cell apoptosis in both *in vitro* cultures and *in vivo* models. Modulation of MAPK signaling ameliorates the inflammatory responses and hepatic cell apoptosis in DENV infection. This accumulation of published data relative to the role of MAPK signaling in inflammatory responses and cell apoptosis in DENV infection is elucidatory, and may help to accelerate the development of novel or repositioned therapies to treat this unpredictable and often debilitating disease.

1. Introduction

Dengue virus (DENV) infection, which is most prevalent in tropical countries, is one of the most important arbo-viral diseases of the 21st century [1]. Four serotypes of DENV are recognized based on antigenicity, and each causes clinical disease [2]. However, multiple strains of each serotype have been identified among outbreaks that developed in endemic areas. DENV-infected patients show different levels of clinical severity, including mild fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), the last of which is the most severe. The hemorrhagic form of DENV infection includes plasma leakage and hematologic disorders. Secondary DENV infection may lead to shock [3] and multiple organ injuries [4,5].

In 1964, Nelson and colleagues first noted the association between DENV infection and thrombocytopenia [6–8]. The hematologic aspects of dengue infections were well-reviewed in 1982 by Halsted, et al. [9]. Abnormalities in hematologic parameters were also observed in animal models, with thrombocytopenia being consistently and prominently reported [10,11]. Boonpucknavig, et al. detected antigens of DENV, and described them as irregular granules in reticuloendothelial cells of the liver, lymph nodes, and spleen of female mice infected with DENV [12]. Later, clinical manifestations of DENV infection that correlated with liver injury were investigated. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT) levels were reported to be abnormal in DENV-infected patients, and AST was significantly higher than ALT [13].

Apoptotic cell death and phagocytic cell activation were preliminarily given as the explanations for DENV-induced liver injury, with the associated importance of viral replication being thereafter reported [14–16]. The pathologic highlights of liver injury in DHF patients were reported from an extensive pathologic study in DENV infection conducted in Myanmar [17]. In that study, livers of DENV-infected patients had the classical signs of cell death in varying degrees, with morphologic changes that included ballooning of hepatocytes, cytoplasmic vacuolization, dilated sinusoids with debris, and Kupffer cell hyperplasia. With clear signs of necrosis, the positive staining of cytoplasmic cleaved caspase-3 in the liver revealed apoptotic cell death. Splenomegaly with dilated splenic sinusoids that contained red blood cells and positive caspase-3 staining were observed [17].

DENV can induce inflammatory response and hepatic cell apoptosis

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JNK1/2 inhibitor reduces dengue virus-induced liver injury

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ABSTRACT

High viral load with liver injury is exhibited in severe dengue virus (DENV) infection. Mitogen activated protein kinases (MAPKs) including ERK1/2 and p38 MAPK were previously found to be involved in the animal models of DENV-induced liver injury. However, the role of JNK1/2 signaling in DENV-induced liver injury has never been investigated. JNK1/2 inhibitor, SP600125, was used to investigate the role of JNK1/2 signaling in the BALB/c mouse model of DENV-induced liver injury. SP600125-treated DENV-infected mice ameliorated leucopenia, thrombocytopenia, hemoconcentration, liver transaminases and liver histopathology. DENV-induced liver injury exhibited induced phosphorylation of JNK1/2, whereas SP600125 reduced this phosphorylation. An apoptotic real-time PCR array profiler was used to screen how SP600125 affects the expression of 84 cell death-associated genes to minimize DENV-induced liver injury. Modulation of caspase-3, caspase-8 and caspase-9 expressions by SP600125 in DENV-infected mice suggests its efficiency in restricting apoptosis via both extrinsic and intrinsic pathways. Reduced expressions of TNF-α and TRAIL are suggestive to modulate the extrinsic apoptotic signals, where reduced p53 phosphorylation and induced anti-apoptotic Bcl-2 expression indicate the involvement of the intrinsic apoptotic pathway. This study thus demonstrates the pivotal role of JNK1/2 signaling in DENV-induced liver injury and how SP600125 modulates this pathogenesis.

1. Introduction

Dengue virus (DENV) infection is one of the most important arbo-viral diseases of the 21st century, most prevalent in tropical and sub-tropical countries (Gubler, 2002). DENV-infected patients show different levels of disease severity; dengue fever, dengue hemorrhagic fever, or the most severe dengue shock syndrome. The hemorrhage signs of DENV infection are represented by plasma leakage and hematologic disorders. Severe infection may lead to hypovolemic shock in DENV infection (Halstead, 2007) with multiple organ injuries (Ghosh et al., 2011; Schmitz et al., 2011).

Liver injury is reported in severe DENV-infected patients (Trung et al., 2010) where apoptosis is evident (Limonta et al., 2007). Elevated alanine transaminase (ALT) and aspartate transaminase (AST) are observed in the patients (Arora et al., 2015; Nguyen et al., 1997; Treeprasertsuk and Kittitrakul, 2015) and animal models (Franca et al., 2010; Paes et al., 2005, 2009; Sreekanth et al., 2016; Sreekanth et al., 2014) of DENV infection. Histopathology correlated with transaminase level is used to understand the severity of liver injury (de Macedo et al., 2006; Huerre et al., 2001). Histopathology of DENV-induced liver injury in Balb/C mice was thoroughly studied (Sakinah et al., 2016). DENV infection induces apoptosis in HepG2 cells (Morchang et al., 2011; Thepparit et al., 2013; Thongtan et al., 2004). DENV infection undergoes apoptosis via activated caspase-8 (Liao et al., 2010), and overexpression of pro-inflammatory cytokines and chemokines including TNF-α, IL-8 and RANTES leads to vascular permeability in DENV-infected patients (Chareonsirisuthigul et al., 2007; Pang et al., 2007). DENV-induced TNF-α mediates apoptosis (Cardier et al., 2005), and TNF-related apoptosis-inducing ligand (TRAIL) modulates type I and

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0166-3542/

PLOS | ONE

RESEARCH ARTICLE

SB203580 Modulates p38 MAPK Signaling and Dengue Virus-Induced Liver Injury by Reducing MAPKAPK2, HSP27, and ATF2 Phosphorylation

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Abstract

Dengue virus (DENV) infection causes organ injuries, and the liver is one of the most important sites of DENV infection, where viral replication generates a high viral load. The molecular mechanism of DENV-induced liver injury is still under investigation. The mitogen activated protein kinases (MAPKs), including p38 MAPK, have roles in the hepatic cell apoptosis induced by DENV. However, the *in vivo* role of p38 MAPK in DENV-induced liver injury is not fully understood. In this study, we investigated the role of SB203580, a p38 MAPK inhibitor, in a mouse model of DENV infection. Both the hematological parameters, leucopenia and thrombocytopenia, were improved by SB203580 treatment and liver transaminases and histopathology were also improved. We used a real-time PCR microarray to profile the expression of apoptosis-related genes. Tumor necrosis factor α, caspase 9, caspase 8, and caspase 3 proteins were significantly lower in the SB203580-treated DENV-infected mice than that in the infected control mice. Increased expressions of cytokines including TNF-α, IL-6 and IL-10, and chemokines including RANTES and IP-10 in DENV infection were reduced by SB203580 treatment. DENV infection induced the phosphorylation of p38MAPK, and its downstream signals including MAPKAPK2, HSP27 and ATF-2. SB203580 treatment did not decrease the phosphorylation of p38 MAPK, but it significantly reduced the phosphorylation of MAPKAPK2, HSP27, and ATF2. Therefore, SB203580 modulates the downstream signals to p38 MAPK and reduces DENV-induced liver injury.

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Role of ERK1/2 signaling in dengue virus-induced liver injury

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ABSTRACT

The liver is considered to be an important organ of dengue virus (DENV) replication and pathogenesis. However, molecular mechanisms of hepatic injury are still poorly understood. Modulation of Mitogen Activated Protein Kinases (MAPKs) was previously shown to affect DENV-induced apoptosis of hepatocytes *in vitro*. However, the *in vivo* role of ERK1/2, a member of the MAPK family, and the question whether its activation can facilitate cell survival or cell death, has not been thoroughly investigated. Therefore, the role of ERK1/2 in a mouse model of DENV infection was examined. Our results show that DENV induces phosphorylation of ERK1/2 and increases apoptosis. Inhibition of phosphorylated ERK1/2 by the selective ERK1/2 inhibitor, FR180204, limits hepatocyte apoptosis and reduces DENV-induced liver injury. Clinical parameters, including leucopenia, thrombocytopenia, transaminases and histology, show improvements after FR180204 treatment. The expression of cell death genes was further identified using real-time PCR array and Western blot analysis. Caspase-3 was significantly decreased in FR180204 treated DENV-infected mice compared to the levels of untreated DENV-infected mice suggesting the role of ERK1/2 signaling in immune-mediated liver injury during DENV infection.

1. Introduction

Dengue virus (DENV) infection is one of the most important mosquito-borne viral diseases, which is endemic in tropical and sub-tropical regions. Clinical manifestations of DENV infection include dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). Patients with DHF generally present with hemorrhagic tendencies, plasma leakage, thrombocytopenia, and hemoconcentration. DSS may occur in cases of subsequent infection with a different serotype of DENV (Halstead, 2007).

Hepatic dysfunction is a crucial feature seen in DENV infection (Halstead, 2007). Transaminase levels are increased in DENV-infected patients (Halstead, 1988; Kuo et al., 1992; Souza et al., 2004) and in a murine model of DENV infection (Barth et al., 2006; Chen et al., 2004; Franca et al., 2010; Paes et al., 2005, 2009). Apoptosis of hepatic cells, which may be related to the pathogenesis of DSS, has been observed both *in vitro* and *in vivo* (Couvelard et al., 1999; El-Bacha et al., 2007; Huerre et al., 2001; Limonta et al., 2007; Lin et al., 2008; Marianneau et al., 1998; Morchang et al., 2011; Nagila et al., 2011, 2013; Netsawang et al., 2010; Thongtan et al., 2004). DENV infection promotes apoptosis in the hepatoma cell line, HepG2, partly through the induction of TRAIL, a member of the death receptor pathway (Matsuda et al., 2005). TNF-α and Fas signaling also contribute to DENV-mediated apoptosis (Limjindaporn et al., 2007; Nagila et al., 2013). The mitochondria of DENV-infected HepG2 cells exhibit functional and morphological defects, suggesting activation of the mitochondrial cell death pathway (El-Bacha et al., 2007). Similarly, DENV infection of Huh-7 cells, another hepatoma cell line, alters mitochondrial function and expression of p53 (Nasirudeen and Liu, 2009; Nasirudeen et al., 2008). Therefore, both virus and cytokines contribute to

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