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Compound A, a dissociated glucocorticoid receptor modulator, reduces dengue virus-induced cytokine secretion and dengue virus production

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ABSTRACT

Dengue Virus (DENV) infection is an important mosquito-borne viral disease and its clinical symptoms range from a predominantly febrile disease, dengue fever (DF), to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Increased levels of cytokines – the so-called 'cytokine storm', contribute to the pathogenesis of DHF/DSS. In this study, we compared the expression of cytokine genes between mock-infected and DENV-infected HepG2 cells using a real-time PCR array and revealed several up-regulated chemokines and cytokines, including CXCL10 and TNF-α. Compound A (CpdA), a plant-derived phenyl aziridine precursor containing anti-inflammatory action and acting as a dissociated nonsteroidal glucocorticoid receptor modulator, was selected as a candidate agent to modulate secretion of DENV-induced cytokines. CpdA is not a glucocorticoid but has an anti-inflammatory effect with no metabolic side effects as steroidal ligands. CpdA significantly reduced DENV-induced CXCL10 and TNF-α secretion and decreased leukocyte migration indicating for the first time the therapeutic potential of CpdA in decreasing massive immune activation during DENV infection.

1. Introduction

Dengue virus (DENV) infection is a mosquito-borne viral disease and is endemic in several countries [1]. Clinical severity of the disease ranges from a predominantly febrile disease, dengue fever (DF), to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. The patients with DHF generally present hemorrhagic tendencies, plasma leakage, thrombocytopenia, and hemoconcentration. Furthermore, hepatic dysfunction is a feature of DENV infection [3,4]. Elevation of aminotransferases and hepatic failure are observed in the patients with DHF/DSS [5].

Optimal levels of cytokines have anti-viral effects during DENV infection [6–8]; however, the massive cytokine secretion following severe DENV infection may contribute to the development of the disease rather than protection [9–12]. Increased levels of cytokines – the so-called 'cytokine storm', which relate to the pathogenesis of severe DENV infection, are observed both *in vitro* and *in vivo* models of DENV infection [13–20].

Compound A (CpdA) or 2-(4-acetoxyphenyl)-2-chloro-*N*-methyl-ethylammonium chloride, from the Namibian shrub *Salsola tuberculatiformis*, is a dissociated glucocorticoid receptor (GR) ligand, which has an anti-inflammatory effect as steroidal ligands but has no metabolic side effects [21–23]. CpdA favors GR monomer formation over GR dimer formation and interferes with NF-κB-driven expression of inflammatory cytokines [21–23]. However, CpdA cannot stimulate glucocorticoid-responsive enhancer elements (GREs) in the target gene promoters. Therefore, the metabolic side effects of steroidal ligands do not occur. CpdA was previously shown to reduce inflammation with less side effects in both mouse models of asthma and autoimmune neuritis [22,24].

In this study, we firstly compared the expression of cytokine genes between mock-infected and DENV-infected HepG2 cells using a real-time PCR array. The highly up-regulated cytokines, including CXCL10 and TNF-α, were subsequently verified at both the mRNA and protein levels. As expected, CpdA significantly reduced DENV-induced CXCL10 and TNF-α secretion indicating for the first time that modulating the immune responses by CpdA

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may have a therapeutic potential in decreasing massive cytokine production during DENV infection.

2. Materials and methods

2.1. Cell culture and infection of HepG2 cells

Up to 2 $\times 10^6$ HepG2 cells were seeded in a 60 mm dish and cultured for 24 h before infection. HepG2 cells were grown in DMEM medium (Gibco-BRL), supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1% non-essential amino acids, 1 mM sodium pyruvate, 100 U/ml of penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. HepG2 cells were washed with PBS and infected with DENV serotype 2 (DENV-2) strain 16881 at a multiplicity of infection (MOI) of 5. Cells were collected at 12, 24, 36 or 48 h post-infection. DENV NS1 antibody staining analysed by flow cytometry demonstrated DENV infection [25]. Cell viability was determined by the trypan blue exclusion test [26].

2.2. Real-time PCR array

Total RNA was isolated either from mock-infected HepG2 cells or from DENV-infected HepG2 cells at 24 h post-infection using the High Pure RNA isolation kit (Roche). RNAs with an OD_{260nm}/OD_{280nm} absorbance ratio of at least 2.0 were used. Total RNA was reverse-transcribed into cDNA using the RT^2 First strand Kit (SA Biosciences), mixed with RT^2 qPCR mastermix containing SYBR Green (SA Biosciences), and aliquoted in equal volumes to each well of the real-time PCR arrays. The Human Cytokine RT^2 Profiler™ PCR Array (SA Biosciences) has 84 genes related to the cytokine pathway. The real-time PCR cycling program was run on a Roche Light Cycler 480. The threshold cycle (Ct) of each gene was determined and subsequently analysed by RT^2 Profiler PCR Array data Analysis software (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php). The highly up-regulated chemokine gene, CXCL10, and cytokine gene, TNF-α, were selected and confirmed at the mRNA level by quantitative Real-time PCR using different sets of primers, including CXCL10 F5′ GAATCGAAGGCCATCAAGAA 3′, CXCL10 R5′ AAGCAGGGTCAGAACATCCA 3′; TNF-αF5′ TGCTTGTTCCTCAGCCTCTT 3′, TNF-α R 5′ ATGGGCTACAGGCTTGTCACT 3′).

2.3. Elisa

The effect of CpdA on CXCL10 and TNF-α production was determined following infection of HepG2 cells with DENV-2 at a MOI of 5 for 2 h, washing and incubation for 24 h in the presence of PBS, 5 μM CpdA, 10 μM CpdA, 20 μM CpdA or 50 μM dexamethasone (Dex) (Sigma Aldrich), respectively. The levels of cytokines were then measured from CpdA-untreated and CpdA-treated DENV-infected HepG2 cells by ELISA (R&D Systems).

2.4. Chemotaxis assay

To test the effect of CpdA on leukocyte migration, the DENV-infected HepG2 cells were treated with CpdA as described in the preceding experiment. The chemotaxis assay was performed using the protocol described previously [27]. Briefly, 600 μl of cell culture supernatant collected from either CpdA-untreated or CpdA-treated DENV-infected HepG2 cells was added to the lower chambers of the transwell cassette (Corning Costar, Lowell, MA, USA). Up to 1 $\times 10^5$ monocyte THP-1 cells were added to the upper chamber and incubated at 37 °C in 5% CO_2 for 6 h. A 500 μl aliquot of the cells migrated to the lower chamber was counted by flow cytometry in a FACCalibur acquiring events for a fixed time period of 60 s using CellQuest software (Becton Dickinson, Basel, Switzerland).

2.5. Focus-forming unit (FFU)

Firstly, viability of mock-infected or DENV-infected HepG2 cells in the presence or absence of CpdA were first measured by trypan blue exclusion test [26]. Secondly, DENV-infected HepG2 cells were treated with CpdA as described in the preceding experiment. Focus-forming unit (FFU) was performed using the protocol described previously [28]. Briefly, titration of DENV was carried out using 96-well plates. Vero cells were seeded at 3 $\times 10^4$ cells/well 24 h prior to infection. Ten-fold serially diluted culture media was added to Vero cells at room temperature (RT) for 2 h. After adsorption, the cells were overlaid with 100 μl of 1.5% gum tragacanth containing 2% FBS in MEM before further incubation at 37 °C for 3 days. The cultures were then fixed with 3.7% formaldehyde for 10 min at RT, treated with 1% Triton X-100 in PBS for another 10 min, washed three times with PBS, and incubated with anti-DENV E (4G2) for 30 min at 37 °C in a humidified chamber. Cells were washed with PBS and incubated with rabbit anti-mouseIgG-HRP at the dilution 1: 1000 for 30 min at 37 °C in a humidified chamber. After triple washing, the substrate solution was added. Stained foci were visible and the reaction was terminated by washing cells with PBS. DENV-infected foci were counted with a light microscope and viral concentration in the supernatant calculated as focus-forming units (FFU) per milliliter.

2.6. Statistical analysis

All data were obtained from three independent experiments and reported as the mean ± SEM. Statistical differences between the groups were tested with an unpaired *t*-test using StatView version 5.0 and *P* value less than 0.05 was considered significant.

3. Results and discussion

3.1. Cytokine expression profile in DENV-infected HepG2 cells

The average percent of HepG2 cells infected with MOI of 5 at 24 h, 36 h and 48 h were 80, 92, and 90, respectively (Fig. 1A). We selected DENV-infected HepG2 cells at 24 h post-infection for accessing changes in expression of cytokine genes as DENV-infected HepG2 cells underwent cell death at 36 h and 48 h post infection (Fig. 1C). Total RNA was prepared from mock-infected HepG2 cells or DENV-infected HepG2 cells and used to probe microarrays. Table 1 and Fig. 2A demonstrated the genes exhibiting a change in cytokine gene expression more than 2-fold. CXCL10 and TNF-α were selected as representatives for DENV-induced cytokines in further analyses, as CXCL10 and TNF-α are the highly up-regulated cytokines in patients with DHF [11,13,19,29,30]. CXCL10 is a potent chemokine elevated during DENV infection. The early induction of CXCL10 during DENV infection plays a role in innate immunity through the recruitment and activation of NK cells [8]. However, intrahepatic infiltrating NK and T cells cause liver cell death in DENV infection [31]. Our results demonstrated that CXCL10 mRNA and protein expression in DENV-infected HepG2 cells are highly increased post DENV infection, respectively (Figs. 2B and 3A and C). Also, TNF-α mRNA and protein expression increased significantly post DENV infection, respectively, in DENV-infected HepG2 cells (Figs. 2C and 3B and D) supporting the massive cytokine secretion followed after severe DENV infection [9–12].

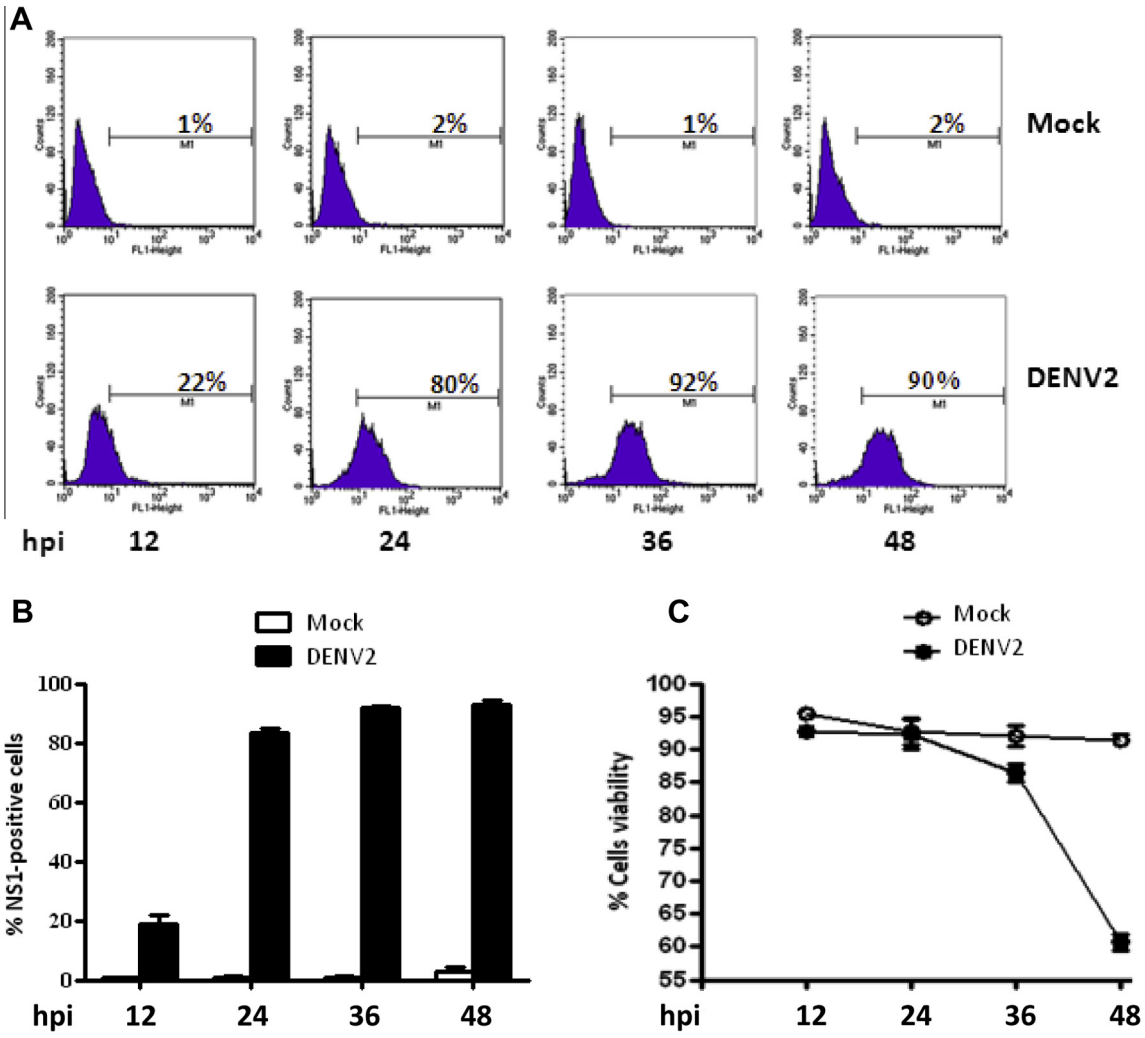

Fig. 1. DENV infection of HepG2 cells. HepG2 cells were infected with DENV serotype 2 at a MOI of 5. DENV NS1 antibody staining was performed at 12, 24, 36 and 48 h post infection and measured by flow cytometry to show DENV infection (A and B). Cell viabilities of mock and DENV-infected HepG2 cells at the indicated times were also determined by trypan blue exclusion test (C). The results are the average of three independent experiments.

3.2. CpdA reduced DENV-induced CXCL10, and TNF-α secretion in HepG2 cells

Proinflammatory cytokines in DENV infection may increase vascular permeability, damage organs, and lead to hypovolumic shock [32]. Glucocorticoids (GCs) are among the most effective anti-inflammatory drugs. Dexamethasone was used to inhibit TNF-α secretion in DENV-infected monocyte cells [33]. However, the side effects of steroidal ligands may deteriorate the patients in clinical setting. We therefore asked whether the dissociated glucocorticoid receptor modulator, CpdA, which has no metabolic side effects as compared to steroidal ligands, would inhibit DENV-induced CXCL10 and TNF-α secretion in HepG2 cells, an approach that might alleviate the DENV-induced cytokine storm. An animal model of DENV-induced liver injury showed the up-regulation of CXCL10. Neutralization of CXCL10 abrogated NK cell recruitment to the liver and diminished liver cell death [31]. In addition, intrahepatic CD8+ cells are cytotoxic against DENV-infected cells [31]. *In vitro* and *in vivo* models implicate TNF-α in DENV-induced tissue damage [12,34,35]. CXCL10 and TNF-α secretion form mock or DENV-infected HepG2 cells were measured in the presence or absence of CpdA. The results showed that CpdA significantly reduced DENV-induced CXCL10 and TNF-α secretion at both mRNA and protein levels in a dose-dependent manner (Fig. 3A–D). The decreased production of CXCL10 by CpdA may decrease immune cell migration, thereby decreasing the cytokine secretion in the DENV-infected liver. We then asked whether CpdA would inhibit DENV-induced leukocyte migration. As expected, CpdA reduced DENV-induced leukocyte migration about 3-fold in a dose-dependent manner (Fig. 4A). Therefore, CpdA may have a therapeutic potential in decreasing massive CXCL10 and TNF-α during DENV infection.

3.3. CpdA reduced DENV production in HepG2 cells

The candidate for a therapeutic agent against DENV should not only decrease massive cytokine production in DENV-infected cells, but should also decrease DENV production in the DENV-infected cells. DENV replication in HepG2 cells activates NF-κB concomitantly with viral protein synthesis, before the appearance of apoptotic cells [36]. We therefore asked whether CpdA, which interferes with NF-κB-driven expression, would inhibit DENV production in HepG2 cells, an approach that might decrease the production of DENV in infected hepatocytes. The results showed that 20 μM of CpdA significantly decreased yield of viral progeny a minimum of 30% and 50 μM of Dex significantly decreased yield of viral progeny a minimum of 70% demonstrating for the first time that CpdA inhibited virus production in HepG2 cells (Fig. 4B). The decreased

Table 1

Gene No.	Fold change	Gene name	Gene description
1	660.77	CXCL10	Chemokine (C-X-C motif) ligand 10
2	191.08	CCL5	Chemokine (C-C motif) ligand 5
3	168.66	TNF-α	Tumor necrosis factor (TNF superfamily, member2)
4	71.41	CCL4	Chemokine (C-C motif) ligand 4
5	44.57	CXCL3	Chemokine (C-X-C motif) ligand 3
6	24.39	CXCL2	Chemokine (C-X-C motif) ligand 2
7	22.91	LTB	Lymphotoxin beta (TNF superfamily, member3)
8	22.75	IL8	Interleukin 8
9	17.85	CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
10	14.01	CXCL5	Chemokine (C-X-C motif) ligand 5
11	11.78	FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog
12	11.46	LTA	Lymphotoxin alpha (TNF superfamily, member1)
13	6.76	IL23A	Interleukin 23, alpha subunit p19
14	4.02	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta
15	3.48	CCL3	Chemokine (C-C motif) ligand 3
16	3.38	CXCL9	Chemokine (C-X-C motif) ligand 9
17	2.84	NOS2A	Nitric oxide synthase 2A (inducible, hepatocytes)
18	2.77	TLR3	Toll-like receptor 3
19	2.46	IL9	Interleukin 9
20	2.23	IL6	Interleukin 6 (interferon beta2)
21	2.16	CCR3	Chemokine (C-C motif) receptor 3
22	2.10	CCL2	Chemokine (C-C motif) ligand 2
23	2.07	TNFSF14	Tumor necrosis factor (ligand) superfamily, member14
24	2.05	IL1F10	Interleukin 1 family, member 10 (theta)

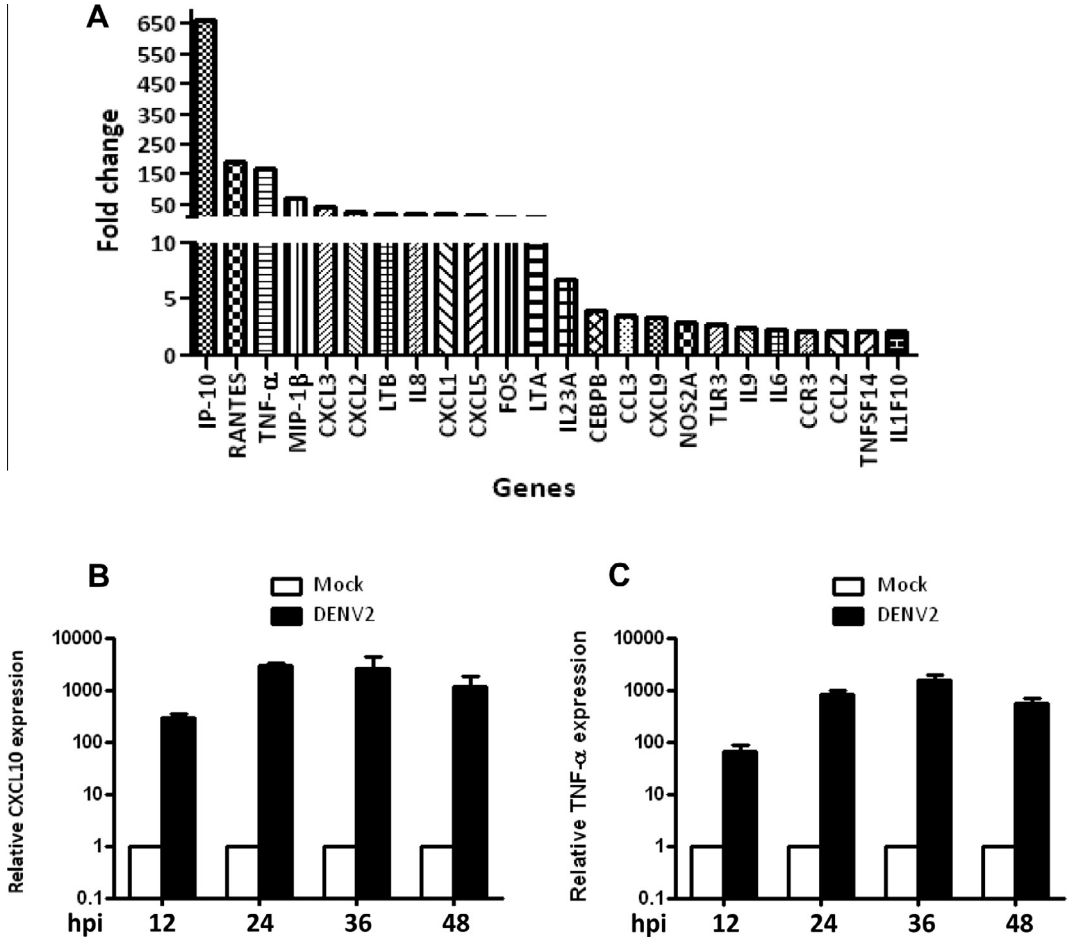

Fig. 2. CXCL10 and TNF-α were among the highly up-regulated cytokines in DENV-infected HepG2 cells. The genes exhibiting a change in cytokine gene expression more than 2-fold was shown in (A). CXCL10 (B) and TNF-α (C) were confirmed at the transcriptional level using different set of primers in DENV-infected HepG2 cells at a MOI of 5 at using real-time PCR at different time points. The results are the average of three independent experiments.

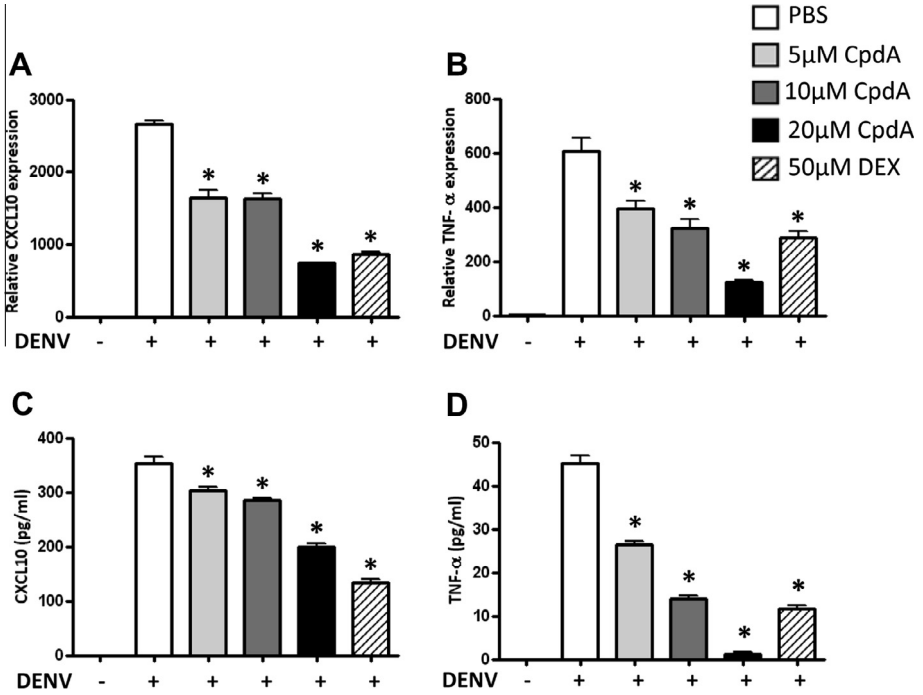

Fig. 3. CpdA reduced DENV-induced CXCL10, and TNF-α secretion. HepG2 cells were infected with DENV at a MOI of 5 and incubated for 24 h in the presence of PBS, 5 μM CpdA, 10 μM CpdA, 20 μM CpdA or 50 μM Dex. The expression levels of CXCL10 and TNF-α were then measured from CpdA-untreated and CpdA-treated DENV-infected HepG2 cells by Real-time PCR (A and B) and ELISA (C and D), respectively. The results are the average of three independent experiments. The asterisks indicate statistically significant differences between samples from CpdA-untreated and CpdA-treated DENV-infected HepG2 cells (*$p < 0.05$).

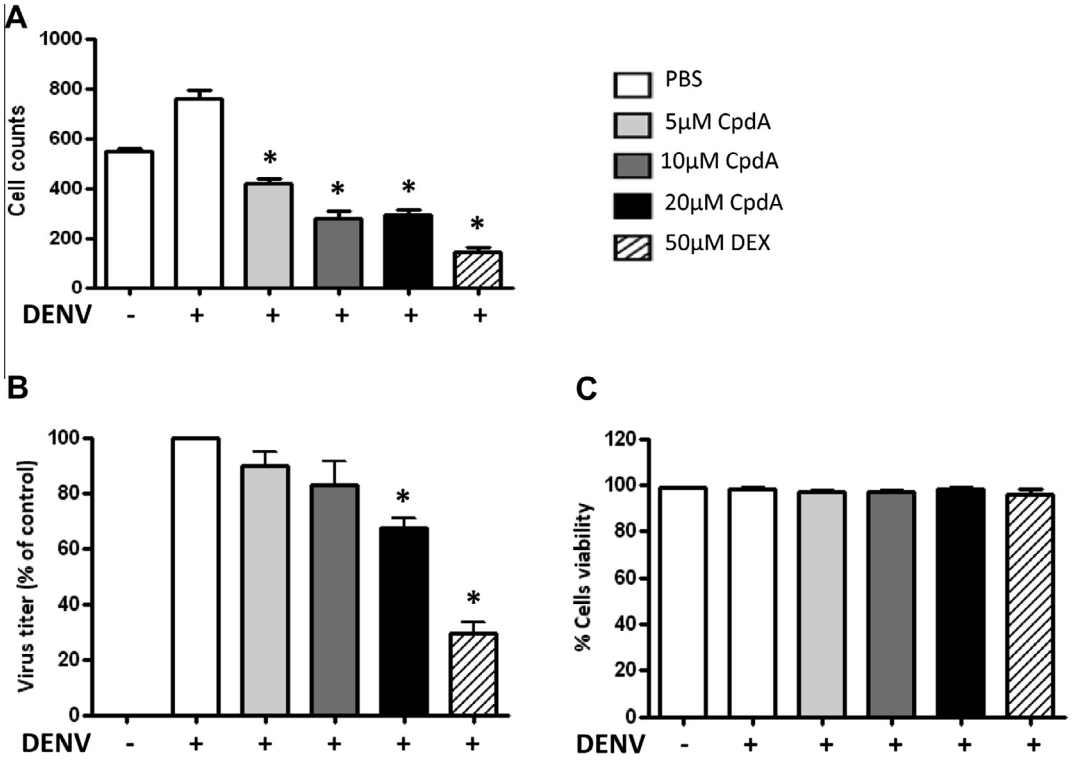

Fig. 4. CpdA reduced the yield of infectious DENV production. HepG2 cells were infected with DENV at a MOI of 5 and incubated for 24 h in the presence of PBS, 5 μM CpdA, 10 μM CpdA, 20 μM CpdA or 50 μM Dex Leukocyte migration was performed using the chemotaxis assay (A). Supernatant was sampled and analyzed for infectious DENV by FFU assay (B). Viability of mock-infected or DENV-infected HepG2 cells in the presence or absence of CpdA was measured by trypan blue exclusion test (C). The results are the average of three independent experiments. The asterisks indicate statistically significant differences between samples from CpdA-untreated and CpdA-treated DENV-infected HepG2 cells (*$p < 0.05$).

DENV production was not from the decreased viable cells as the number of mock-infected and DENV-infected HepG2 cells was similar at the 24 h post infection (Fig. 4C). Similar to our study, addition of reduced glutathione (GSH) to the medium of DENV-infected HepG2 cells was also shown to inhibit activity of NF-κB thereby decreasing DENV production [37]. It is interesting to ask and further investigate why inhibition of NF-kB decreases DENV production.

In summary, this study revealed the up-regulation of several cytokine genes, including CXCL10 and TNF-α, during DENV infection in HepG2 cells. CpdA reduced massive DENV-induced secretion of CXCL10 and TNF-α cytokines, decreased leukocyte migration and decreased DENV production in HepG2 cells. The molecular mechanisms how CpdA inhibits NF-κB signaling in DENV infection merits further investigation.

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References

[1] S.B. Halstead, Dengue, Lancet 370 (2007) 1644–1652.
[2] S.B. Halstead, Pathogenesis of dengue: challenges to molecular biology, Science 239 (1988) 476–481.
[3] C.H. Kuo, D.I. Tai, C.S. Chang-Chien, C.K. Lan, S.S. Chiou, Y.F. Liaw, Liver biochemical tests and dengue fever, Am. J. Trop. Med. Hyg. 47 (1992) 265–270.
[4] L.J. Souza, J.G. Alves, R.M. Nogueira, C. Gicovate Neto, D.A. Bastos, E.W. Siqueira, J.T. Souto Filho, A. Cezario Tde, C.E. Soares, C. Carneiro Rda, Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1585 cases, Braz. J. Infect. Dis. 8 (2004) 156–163.
[5] A. Khongphatthanayothin, P. Lertsapcharoen, P. Supachokchaiwattana, P. Satupan, K. Thongchaiprasit, Y. Poovorawan, C. Thisyakorn, Hepatosplanchnic circulatory dysfunction in acute hepatic infection: the case of dengue hemorrhagic fever, Shock 24 (2005) 407–411.
[6] M.F. Hsieh, S.L. Lai, J.P. Chen, J.M. Sung, Y.L. Lin, B.A. Wu-Hsieh, C. Gerard, A. Luster, F. Liao, Both CXCR3 and CXCL10/IFN-inducible protein 10 are required for resistance to primary infection by dengue virus, J. Immunol. 177 (2006) 1855–1863.
[7] L. Gil, C. Lopez, A. Blanco, L. Lazo, J. Martin, I. Valdes, Y. Romero, Y. Figueroa, G. Guillen, L. Hermida, The cellular immune response plays an important role in protecting against dengue virus in the mouse encephalitis model, Viral Immunol. 22 (2009) 23–30.
[8] J.P. Chen, H.L. Lu, S.L. Lai, G.S. Campanella, J.M. Sung, M.Y. Lu, B.A. Wu-Hsieh, Y.L. Lin, T.E. Lane, A.D. Luster, F. Liao, Dengue virus induces expression of CXC chemokine ligand 10/IFN-gamma-inducible protein 10, which competitively inhibits viral binding to cell surface heparan sulfate, J. Immunol. 177 (2006) 3185–3192.
[9] A. Atrasheuskaya, P. Petzelbauer, T.M. Fredeking, G. Ignatyev, Anti-TNF antibody treatment reduces mortality in experimental dengue virus infection, FEMS Immunol. Med. Microbiol. 35 (2003) 33–42.
[10] R. Guabiraba, R.E. Marques, A.G. Besnard, C.T. Fagundes, D.G. Souza, B. Ryffel, M.M. Teixeira, Role of the chemokine receptors CCR1, CCR2 and CCR4 in the pathogenesis of experimental dengue infection in mice, PLoS One 5 (2010) e15680.
[11] A. Rathakrishnan, S.M. Wang, Y. Hu, A.M. Khan, S. Ponnampalavanar, L.C. Lum, R. Manikam, S.D. Sekaran, Cytokine expression profile of dengue patients at different phases of illness, PLoS One 7 (2012) e52215.
[12] Y.T. Yen, H.C. Chen, Y.D. Lin, C.C. Shieh, B.A. Wu-Hsieh, Enhancement by tumor necrosis factor alpha of dengue virus-induced endothelial cell production of reactive nitrogen and oxygen species is key to hemorrhage development, J. Virol. 82 (2008) 12312–12324.
[13] D. Hober, L. Poli, B. Roblin, P. Gestas, E. Chungue, G. Granic, P. Imbert, J.L. Pecarere, R. Vergez-Pascal, P. Wattre, et al., Serum levels of tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 beta) in dengue-infected patients, Am. J. Trop. Med. Hyg. 48 (1993) 324–331.
[14] P. Avirutnan, P. Malasit, B. Seliger, S. Bhakdi, M. Husmann, Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis, J. Immunol. 161 (1998) 6338–6346.
[15] C.P. Simmons, S. Popper, C. Dolocek, T.N. Chau, M. Griffiths, N.T. Dung, T.H. Long, D.M. Hoang, N.V. Chau, T.T. Thao le, T.T. Hien, D.A. Relman, J. Farrar, Patterns of host genome-wide gene transcript abundance in the peripheral blood of patients with acute dengue hemorrhagic fever, J. Infect. Dis. 195 (2007) 1097–1107.
[16] D.H. Libraty, T.P. Endy, H.S. Houng, S. Green, S. Kalayanarooj, S. Suntayakorn, W. Chansiriwongs, D.W. Vaughn, A. Nisalak, F.A. Ennis, A.L. Rothman, Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections, J. Infect. Dis. 185 (2002) 1213–1221.
[17] Y.L. Lin, C.C. Liu, J.I. Chuang, H.Y. Lei, T.M. Yeh, Y.S. Lin, Y.H. Huang, H.S. Liu, Involvement of oxidative stress, NF-IL-6, and RANTES expression in dengue-2-virus-infected human liver cells, Virology 276 (2000) 114–126.
[18] L. Suksanpaisan, A. Cabrera-Hernandez, D.R. Smith, Infection of human primary hepatocytes with dengue virus serotype 2, J. Med. Virol. 79 (2007) 300–307.
[19] J. Fink, F. Gu, L. Ling, T. Tolfvenstam, F. Olfat, K.C. Chin, P. Aw, J. George, V.A. Kuznetsov, M. Schreiber, S.G. Vasudevan, M.L. Hibberd, Host gene expression profiling of dengue virus infection in cell lines and patients, PLoS Negl. Trop. Dis. 1 (2007) e86.
[20] T.M. Conceicao, T. El-Bacha, C.S. Villas-Boas, G. Coello, J. Ramirez, M. Montero-Lomeli, A.T. Da Poian, Gene expression analysis during dengue virus infection in HepG2 cells reveals virus control of innate immune response, J. Infect. 60 (2009) 65–75.
[21] K. De Bosscher, W. Vanden Berghe, I.M. Beck, W. Van Molle, N. Hennuyer, J. Hapgood, C. Libert, B. Staels, A. Louw, G. Haegeman, A fully dissociated compound of plant origin for inflammatory gene repression, Proc. Natl. Acad. Sci. USA 102 (2005) 15827–15832.
[22] L.L. Reber, F. Daubeuf, M. Plantinga, L. De Cauwer, S. Gerlo, W. Waelput, S. Van Calenbergh, J. Tavernier, G. Haegeman, B.N. Lambrecht, N. Frossard, K. De Bosscher, A dissociated glucocorticoid receptor modulator reduces airway hyperresponsiveness and inflammation in a mouse model of asthma, J. Immunol. 188 (2012) 3478–3487.
[23] P. Dewint, V. Gossye, K. De Bosscher, W. Vanden Berghe, K. Van Beneden, D. Deforce, S. Van Calenbergh, U. Muller-Ladner, B. Vander Cruyssen, G. Verbruggen, G. Haegeman, D. Elewaut, A plant-derived ligand favoring monomeric glucocorticoid receptor conformation with impaired transactivation potential attenuates collagen-induced arthritis, J. Immunol. 180 (2008) 2608–2615.
[24] Z. Zhang, Z.Y. Zhang, H.J. Schluesener, Compound A, a plant origin ligand of glucocorticoid receptors, increases regulatory T cells and M2 macrophages to attenuate experimental autoimmune neuritis with reduced side effects, J. Immunol. 183 (2009) 3081–3091.
[25] S. Noisakran, S. Sengsai, V. Thongboonkerd, R. Kanlaya, S. Sinchaikul, S.T. Chen, C. Puttikhunt, W. Kasinrerk, T. Limjindaporn, W. Wongwiwat, P. Malasit, P.T. Yenchitsomanus, Identification of human hnRNP C1/C2 as a dengue virus NS1-interacting protein, Biochem. Biophys. Res. Commun. 372 (2008) 67–72.
[26] W. Strober, Trypan blue exclusion test of cell viability. Curr. Protoc. Immunol. Appendix 3 (2001) Appendix 3B.
[27] E. Scandella, Y. Men, D.F. Legler, S. Gillessen, L. Prikler, B. Ludewig, M. Groettrup, CCL19/CCL21-triggered signal transduction and migration of dendritic cells requires prostaglandin E2, Blood 103 (2004) 1595–1601.
[28] N. Jirakanjanakit, T. Sanohsomneing, S. Yoksan, N. Bhamarapravati, The micro-focus reduction neutralization test for determining dengue and Japanese encephalitis neutralizing antibodies in volunteers vaccinated against dengue, Trans. R. Soc. Trop. Med. Hyg. 91 (1997) 614–617.
[29] W. Dejnirattisai, T. Duangchinda, C.L. Lin, S. Vasanawathana, M. Jones, M. Jacobs, P. Malasit, X.N. Xu, G. Screaton, J. Mongkolsapaya, A complex interplay among virus, dendritic cells, T cells, and cytokines in dengue virus infections, J. Immunol. 181 (2008) 5865–5874.
[30] G.N. Malavige, L.C. Huang, M. Salimi, L. Gomes, S.D. Jayaratne, G.S. Ogg, Cellular and cytokine correlates of severe dengue infection, PLoS One 7 (2012) e50387.
[31] J.M. Sung, C.K. Lee, B.A. Wu-Hsieh, Intrahepatic infiltrating NK and CD8 T cells cause liver cell death in different phases of dengue virus infection, PLoS One 7 (2012) e46292.
[32] S. Green, A. Rothman, Immunopathological mechanisms in dengue and dengue hemorrhagic fever, Curr. Opin. Infect. Dis. 19 (2006) 429–436.
[33] S.R. Reis, A.L. Sampaio, M. Henriques, M. Gandini, E.L. Azeredo, C.F. Kubelka, An in vitro model for dengue virus infection that exhibits human monocyte infection, multiple cytokine production and dexamethasone immunomodulation, Mem. Inst. Oswaldo Cruz 102 (2007) 983–990.
[34] S. Wati, S.M. Rawlinson, R.A. Ivanov, L. Dorstyn, M.R. Beard, D.A. Jans, S.M. Pitson, C.J. Burrell, P. Li, J.M. Carr, Tumour necrosis factor alpha (TNF-{alpha}) stimulation of cells with established dengue virus type 2 infection induces cell death that is accompanied by a reduced ability of TNF-{alpha} to activate nuclear factor {kappa}B and reduced sphingosine kinase-1 activity, J. Gen. Virol. 92 (2011) 807–818.
[35] A. Nagila, J. Netsawang, A. Suttitheptumrong, A. Morchang, S. Khunchai, C. Srisawat, C. Puttikhunt, S. Noisakran, P.T. Yenchitsomanus, T. Limjindaporn, Inhibition of p38MAPK and CD137 signaling reduce dengue virus-induced TNF-alpha secretion and apoptosis, Virol. J. 10 (2013) 105.
[36] P. Marianneau, A. Cardona, L. Edelman, V. Deubel, P. Despres, Dengue virus replication in human hepatoma cells activates NF-kappaB which in turn induces apoptotic cell death, J. Virol. 71 (1997) 3244–3249.
[37] Y. Tian, W. Jiang, N. Gao, J. Zhang, W. Chen, D. Fan, D. Zhou, J. An, Inhibitory effects of glutathione on dengue virus production, Biochem. Biophys. Res. Commun. 397 (2010) 420–424.