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Short communication

Heparin inhibits dengue-2 virus infection of five human liver cell lines

Yin-Ling Lin [a], Huan-Yao Lei [b], Yee-Shin Lin [b], Trai-Ming Yeh [b], Shun-Hua Chen [b], Hsiao-Sheng Liu [b,*]

[a] *Department of Microbiology and Immunology and The Walther Oncology Center, The Walther Cancer Institute, Indiana University School of Medicine, Indianapolis, IN, USA*

[b] *Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan, ROC*

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Abstract

Liver is suggested to be the major target of dengue virus infection and plays an important role in the immunopathogenesis of dengue hemorrhagic fever. Previously, we reported that five human liver cell lines (HuH-7, HA22T, Hep3B, PLC, and Chang liver) with various degrees of differentiation and tumorigenicity showed different susceptibility for dengue virus infection. Here, we demonstrate that heparin, an analogue of heparan sulfate (HS), can compete with HS on cell membrane for virus binding and subsequently inhibits the replication of dengue-2 and Japanese encephalitis viruses in hepatoma and BHK-21 cells, respectively. It indicates that the binding of these viruses with HS is an important process for their invasion. Moreover, the inhibitory effect of heparin correlates with the infectivity of the virus in the cells. All together, our results suggest that HS is an important host component for dengue and Japanese encephalitis virus replication, which can be effectively blocked by heparin.

Keywords: Dengue virus; Liver cells; Heparin

Patients with dengue virus infection show a wide range of clinical symptoms, from mild fever to life-threatening hemorrhagic fever and/or shock syndrome (DHF/DSS) (Halstead, 1989). Clinical observations and mouse models have confirmed that the liver is the site of dengue virus pathogenesis and might be one of the important targets for dengue virus infection (Halstead, 1989; An et al., 1999; Couvelard et al., 1999). In addition, we have demonstrated that dengue-2 virus can replicate and cause evident cytopathic effects (CPE) in different liver cell lines (Lin et al., 2000c).

Binding of virus to the target cell is a critical factor in determining the tissue tropism and pathogenesis of the virus. It has been reported that dengue virus binds to heparan sulfate (HS), a highly sulfated glycosaminoglycans, as a high affinity receptor on the cell membrane before entering the cells (Chen et al., 1997; Marks et al.,

* Corresponding author. Tel.: +886-6-235-3535x5630; fax: +886-6-208-2705

E-mail address: a713@mail.ncku.edu.tw (H.-S. Liu).

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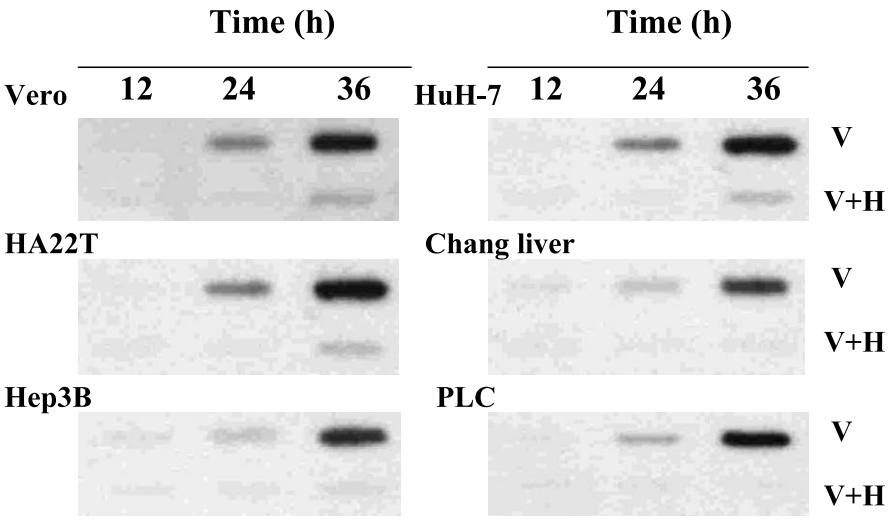

Fig. 1. Heparin inhibits dengue-2 virus infection of five human liver cell lines. Dengue-2 virus (m.o.i. = 100) mixed with 100 μg/ml of heparin was used to infect Vero cells (ATCC CCL 81) and five liver cell lines (1×10^5) including HA22T (Lin et al., 1982), Hep3B (ATCC HB-8064), HuH-7 (Nakabayashi et al., 1982), Chang liver (ATCC CCL 13) and PLC (MacNab et al., 1976). Total cellular RNA was extracted for viral (−) RNA detection by slot blotting at 12, 24 and 36 h p.i. Glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as the internal control. V: dengue-2 virus; V+H: dengue-2 virus mixed with heparin.

2001). HuH-7, a human hepatoma cell line, was also demonstrated to be dependent on HS for the initiation binding with dengue viruses (Hilgard and Stockert 2000). However, an inconsistency about the necessity of HS for dengue virus was observed (Beielefeldt-Ohmann et al., 2001). Previously, we found that dengue-2 virus had higher replication rates in HuH-7, PLC, Hep3B, and Chang liver cells, and lower replication rate in the de-differentiated HA22T cells (Lin et al., 2000c). Chang liver cell is a non-malignant liver epithelial cell line of human origin (Matsuguchi et al., 1990), and the others are human hepatoma cell lines. To examine HS as a potential receptor for dengue virus binding to the liver cells, we used heparin, which is structurally similar to heparan sulfate, to compete with dengue viruses for binding to cells. Five liver cell lines were infected with dengue-2 virus (PL0146 isolated from a patient in Taiwan) (m.o.i. = 100) on ice for 10 min in the presence of heparin (100 μg/ml) (Chen et al., 1997). The infected cells were washed three times with PBS and cultured in DMEM containing 10% FBS. Total cellular RNA was extracted at 12, 24 and 36 h p.i. for viral (−) strand RNA detection by slot blot analysis. A 419 bp probe representing dengue-2 virus sequence from 3009 to 3428 (Henchal et al., 1991) was labeled by asymmetry PCR labeling (Lin et al., 2000b). Glyceraldehydes-3-phosphate dehydrogenase (GAPDH) which is constitutively expressed in cells was used as the internal control (Lin et al., 2000b). As shown in Fig. 1, heparin treatment can effectively inhibit dengue-2 virus replication in five human liver cell lines as well as in Vero monkey cell lines at 12, 24 and 36 h p.i.. The RNA band intensity from the heparin-treated sample was dramatically decreased as compared to the untreated infection, although the band was not quantified. The same blot was striped and then hybridized with GAPDH probe to confirm equal loading (data not shown). These data indicate that dengue-2 virus utilized heparan sulfate to infect these cells. We also found that heparin has the ability to inhibit dengue-2 virus infection of the endothelial cells ECN304 (data not shown). Collectively, these data strongly support the notion that dengue-2 virus enters host cells through HS.

To understand the specificity of heparin inhibition, four types of dengue viruses were examined. Heparin at various dosages (0.1, 1, 10 and 100 μg/ml) was mixed with the viruses and used to infect 2×10^5 of baby hamster kidney cells (BHK-21).

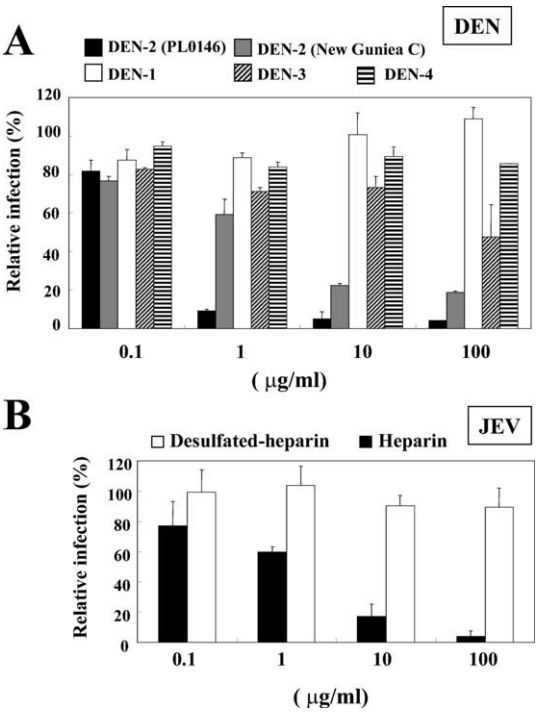

Fig. 2. Heparin inhibits dengue-2 virus and JEV infection, but not dengue-1, -3 and -4 viruses. Different doses of heparin or DS-heparin (0.1, 1, 10 and 100 μg/ml) were mixed with the indicated viruses at 100 m.o.i. to infect BHK-21 cells (ATCC CCL 10) in the 24-well tray. At 48 h p.i., the culture supernatant was harvested for plaque assay. The percentage of virus infection was calculated by dividing the plaque number of the well in the presence of heparin with the plaque number in the absence of heparin. (A) Comparing the inhibitory effects of heparin on different dengue viruses including DEN-1 (766733), DEN-2 PL0146, DEN-2 (New Guinea C), DEN-3 (739079), and DEN-4 (H-241). (B) Comparing the inhibitory effects of heparin and desulfated-heparin on the infection of JEV.

Viral titer was determined by plaque assay from the harvested culture medium 48 h p.i. (Lin et al., 2000a). Fig. 2A, demonstrates that the titer of the dengue-2 virus PL0146 strain was significantly reduced by heparin treatment (IC_{50}: 0.3 μg/ml), however, the titer of dengue-2 virus New Guinea C strain was reduced to a lesser extent (IC_{50}: 3 μg/ml). The suppression level of heparin on dengue virus type 1, 3 and 4 was less significant compared to dengue virus type 2 (Fig. 2A) and this seems to be consistent with poor virus replication in the cells (data not shown). Japanese encephalitis virus (JEV), a member of the Flaviviridae family, has been shown to require HS for viral entry (Su et al., 2001). We also tested whether heparin could inhibit the infection of JEV, which showed very good infectivity in the cultured cells (data not shown). Fig. 2B shows that heparin inhibited JEV infection in a dose-dependent fashion. Moreover, when the sulfate was removed from heparin (DS-heparin), the inhibitory effect was abolished. This result clearly demonstrates the importance of sulfate moiety for the inhibition of heparin (Fig. 2B).

Dengue-2 virus New Guinea C strain showed lower virus replication rate in tissue culture as compared to the Taiwan strain PL0146, this decrease in replication correlates with a decrease in heparin inhibition. Similarly, dengue-1, -3 and -4 viruses, which were isolated from patients and replicated poorly in cells, were less effectively inhibited by heparin (data not shown). It has been reported that foot-and mouth disease virus (FMDV) becomes more virulent after repeated culturing in BHK cells and correspondingly, the potential to bind heparin also increased (Escarmis et al., 1998). Similar mutations are found in the envelope proteins of Sindbis virus (Klimstra et al., 1998). Thus, the adaptive mutations of the virus may increase its binding to cell surface HS in order to efficiently infect cultured cells. Viruses bound to heparin may attenuate its virulence in animal models (Byrnes and Griffin, 2000). Thus, heparin binding can potentially be utilized as a screening tool to select mutant virus for future studying in animal. Collectively, we have demonstrated that heparin can inhibit dengue-2 virus invasion in different liver cells. Also, we have shown the different susceptibility of different types of dengue viruses to heparin inhibition. Overall, these data increase our understanding of the interaction between the virus and the target cell.

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References

An, J., Kimura-Kuroda, J., Hirabayashi, Y., Yasui, K., 1999. Development of a novel mouse model for dengue virus infection. Virology 263, 70–77.

Beielefeldt-Ohmann, H., Meyer, M., Fitzpatrick, D.R., Mackenzie, J.S., 2001. Dengue virus binding to human leukocyte cell lines: receptor usage differs between cell types and virus strains. Virus Res. 73, 81–89.

Byrnes, A.P., Griffin, D.E., 2000. Large-plaque mutants of Sindbis virus show reduced binding to heparan sulfate, heightened viremia, and slower clearance from the circulation. J. Virol. 74, 644–651.

Chen, Y., Maguire, T., Hileman, R.E., Fromm, J.R., Esko, J.D., Linhardt, R.J., Marks, R.M., 1997. Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. Nat. Med. 3, 866–871.

Couvelard, A., Marianneau, P., edel, C., Drouet, M.T., Vachon, F., Henin, D., Deubel, V., 1999. Report of a fatal case of dengue infection with hepatitis: demonstration of dengue antigens in hepatocytes and liver apoptosis. Hum. Pathol. 30, 1106–1110.

Escarmis, C., Carrillo, E.C., Ferrer, M., Garcia Arriaza, J.F., Lopez, N., Tami, C., Verdaguer, N., Domingo, E., Franze-fernandez, M.T., 1998. Rapid selection in modified BHK-21 cells of a foot-and-mouth disease virus variant showing alterations in cell tropism. J. Virol. 72, 10171–10179.

Halstead, S.B., 1989. Antibody, macrophages, dengue virus infection, shock, and hemorrhage: a pathogenic cascade. Rev. Infect. Dis. 11, S830–839.

Henchal, E.A., Polo, S.L., Vorndam, V., Yaemsiri, C., Innis, B., Hoke, C.H., 1991. Sensitivity and specificity of a universal primer set for the rapid diagnosis of dengue virus infections by polymerase chain reaction and nucleic acid hybridization. Am. Soc. Trop. Med. Hyg. 45, 418–428.

Hilgard, P., Stockert, R., 2000. Heparan sulfate proteoglycans initiate dengue virus infection of hepatocytes. Hepatology 32, 1069–1077.

Klimstra, W.B., Ryman, K.D., Johnston, R.E., 1998. Adaptation of sindbis virus to BHK cells selects for use of heparan sulfate as an attachment receptor. J. Virol. 72, 735–736.

Lin, Y.M., Hu, C.P., Chou, C.K., O-Lee, T.W., Wuu, K.T., Chen, T.Y., Peng, F.K., Liu, T.J., Ko, J.L., Chang, C.M., 1982. A new human hepatoma cell line: establishment and characterization. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi. 15, 193–201.

Lin, Y.L., Lei, H.Y., Wen, Y.Y., Luh, T.Y., Chou, C.K., Liu, H.S., 2000a. Light-independent inactivation of Dengue-2 virus by carboxyfullerene C3 isomer. Virology 275, 258–262.

Lin, Y.L., Liu, C.C., Chuang, J.I., Lei, H.Y., Yeh, T.M., Lin, Y.S., Huang, Y.H., Liu, H.S., 2000b. Involvement of oxidative stress, NF-IL-6 and RANTES expression in dengue-2 virus infected human liver cells. Virology 276, 114–126.

Lin, Y.L., Liu, C.C., Lei, H.Y., Yeh, T.M., Lin, Y.S., Chen, R.M.Y., Liu, H.S., 2000c. Infection of five human liver cell lines by dengue-2 virus. J. Med. Virol. 60, 425–431.

MacNab, G.M., Alexander, J.J., Lecatsas, G., Bey, E.M., Urbanowicz, J.M., 1976. Hepatitis B surface antigen produced by a human hepatoma cell line. Br. J Cancer 34, 509–515.

Marks, R.M., Lu, H., Sundaresan, R., Toida, T., Suzuki, A., Imanari, T., Hernaiz, M.J., Linhardt, R.J., 2001. Probing the interaction of dengue virus envelope protein with heparin: assessment of glycosaminoglycan-derived inhibitors. J. Med. Chem. 44, 2178–2187.

Matsuguchi, T., Okamura, S., Kawasaki, C., Niho, Y., 1990. Production of interleukin 6 from human liver cell lines: production of interleukin 6 is not concurrent with the production of alpha-fetoprotein. Cancer Res. 50, 7457–7459.

Nakabayashi, H., Taketa, K., Miyano, K., Yamane, T., Sato, J., 1982. Growth of human hepatoma cells lines with differentiated functions in chemically defined medium. Cancer Res. 42, 3858–3863.

Su, C.-M., Liao, C.-L., Lee, Y.-L., Lin, Y.-L., 2001. Highly sulfated forms of heparin sulfate are involved in Japanese encephalitis virus infection. Virology 286, 206–215.