Effect of Dengue Virus Infection on The Permeability of Vero Cells line

by Nk Kali

Submission date: 30-Jun-2023 04:24AM (UTC-0500)
Submission ID: 2124700810
File name: 1.4958474_AIP.pdf (450.23K)
Word count: 2820
Character count: 14338

Effect of dengue virus infection on the permeability of vero cells line

Nur Kaliwantoro, Marsetyawan Hne Soesatyo, Indarto, Mohammad Juffrie, and Rini Dharmastiti

Citation: AIP Conference Proceedings **1755**, 030003 (2016); doi: 10.1063/1.4958474
View online: http://dx.doi.org/10.1063/1.4958474
View Table of Contents: http://scitation.aip.org/content/aip/proceeding/aipcp/1755?ver=pdfcov
Published by the AIP Publishing

Effect of Dengue Virus Infection on The Permeability of Vero Cells line

Nur Kaliwantoro[1,a)], Marsetyawan HNE Soesatyo[2,b)], Indarto[3,c)], Mohammad Juffrie[4,d)] and Rini Dharmastiti[5,e)]

[1]*Biomedical Engineering, Graduate School, Gadjah Mada University*
Jl. Teknika Utara, Pogung, Yogyakarta, Indonesia
[2]*Histology and Cells Biology Department, Faculty of Medicine, Gadjah Mada University*
Jl. Farmako, Sekip, Yogyakarta, Indonesia
[3]*Mechanical Engineering Department, Faculty of Engineering, Gadjah Mada University*
Jl. Grafika No. 2, Yogyakarta, Indonesia
[4]*Pediatric Department, Faculty of Medicine, Gadjah Mada University*
Jl. Farmako, Sekip, Yogyakarta, Indonesia
[5]*Industrial Engineering Department, Faculty of Engineering, Gadjah Mada University*
Jl. Grafika No. 2, Yogyakarta, Indonesia

[a)]Corresponding author: kaliwantoro@yahoo.com
[b)]marshnes@ugm.ac.id, [c)]indarto@ugm.ac.id, [d)]juffrie@indosat.net.id, [e)]rini@ugm.ac.id,

Abstract. Vero cell line is widely used in many in vitro studies including dengue infection studies. Recent study explore the increase of permeability of vero cell line due to infection dengue virus serotype 2. Vero cell line (CCL-81, ATCC) cultured in the transwell polyester permeable membrane and infected by dengue virus-2. Culture media containing albumin was used to assess the transvero albumin permeability. The content of albumin migrates across the vero cells was determined using protein assay dye reagent and BSA standard (5 and 10 µg/µl), and then measured by microplate reader. The results indicate that dengue infection increases the permeability of vero cells with significant value compared to the control, especially at two days and more post infection. The result of the study suggests that vero cell line can be used as model cells in the permeability studies of infection of dengue virus serotype 2.

INTRODUCTION

Dengue is the most wide spread mosquito-borne disease with around 50 million cases annually [1]. World Health Organization [2] estimates that 500.000 cases need hospitalization and 2.5% of them are fatal. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, in the present decade, from urban to rural regions [2]. Dengue occurs mainly in South and South East Asia, Central and South America, Africa, Carribean and Pacific regions and is endemic in more than 100 countries.

In many cases of death in dengue are characterized by plasma leakage caused by the increase of acute vascular permeability due to disfunction of endothelial cells infected by dengue virus [2]. It was revealed that 91% of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) patients have spontaneous bleeding manifestation and 79% of them have pleura effusion. Cardier et al [4] reported the presence of circulating endothelial cells in the peripheral blood as the evidence of vascular damage in DHF patients. An autopsy study of 63 years old male patient died due to DHF revealed tissue damaged, caused by intense haemorrhage, interstitial edema and inflammation. Viral antigen was found in inflammatory cells of liver, lung, heart, kidney and lymph nodes, as well as in hepatocytes and endothelial cells [5].

Advances of Science and Technology for Society
AIP Conf. Proc. 1755, 030003-1–030003-5; doi: 10.1063/1.4958474
Published by AIP Publishing. 978-0-7354-1413-6/$30.00

The pathogenesis of plasma leakage in dengue infection is not clearly understood due to its difficulties to performed any in vivo studies directly to DSS patients. Thus, many researchers use in vitro models to explore the pathogenesis of plasma leakage in dengue infection [6,7]

Besides endothelial cells, vero cell is the standard model cells that commonly used in dengue studies. The vero lineage was epithelial cells isolated from the kindney of African green monkey (*Chlorocebus* sp) [8]. The infectability of Vero cell line is higher than the endothelial cells, and slightly lower than the monocytes [9]. Another research found that the epithelial cells can expresses many cytokines/chemokines in respon to dengue infection as found in endothelial cells [10]. In turn, cytokines mediate changes in paracellular permeability of the endothelial and epithelial cells, two types of cells that serve as barriers to diffusion of solutes between body compartements [11]. Although epithelial cells can release cytokines in response to dengue infection, the observation of the increase of permeability of dengue infected-vero cell has not been performed yet. Recent study explore the effect of dengue virus serotype-2 on the permeability of vero cell cultured on polyester permeable membrane.

MATERIALS AND METHODS

Culturing Vero Cell Line on The Transwell Polyester Permeable Membrane

Vero cell line (CCL-81, ATCC) were availabled at the Parasitology Laboratory, Medical Faculty of Gadjah Mada University, Indonesia. Culture method of vero cell line (CC-81, ATCC) on the transwell polyester permeable membrane referred to the method as described by previous researchs [7,12] with minor modification to prevent culture media leakage. Vero cells were grown on the 24 mm transwells polyester permeable membrane with pore size 3 µm (Corning cat. 3452) using complete media contains M199 and 10% FBS. To reduce cells escape through the relatively large pore size permeable membrane, transwells containing vero cells were put into the 10 cm petridish first, and tightly closed using its lid and then sent to CO_2 incubator for two days. On the third day, the transwells were moved into 6 well plate. An appropriate culture media was then added into subluminal and luminal of the transwell, and then sent to CO_2 incubator until reached confluency. The media was replaced every three days.

Infection of Vero cells Line with Dengue Virus

Dengue Virus infection on the vero cell culture referred to the method of Beti [7] with minor modification. Culture media in the transwell containing confluent vero cells was removed and washed three times using PBS. 50 µl media contains DV-2 was added into each transwell and incubated for 2 hours at 37ºC and 5% CO_2.

Transvero Albumin Permeability

Transwells containing vero cells incubated by dengue virus-2 for two hours were washed using PBS without $CaCl_2$ and $MgCl_2$ [PBS(-)]. 2 ml of complete medium contains 10 mg/ml BSA (bovine serum albumin (Gibco)) was added into the upper chamber of each transwell polyester membrane culture dish containing vero cell culture. The transwells were then sent back into the CO_2 incubator at 37ºC and 5% CO_2. After 24 hours in the CO_2 incubator, the amount of BSA migrated to the lower chamber of the transwell membrane was collected and repeated after 48 and 72 hours. The content of albumin migrates across the vero cells was determined using Bio-Rad protein assay dye reagent and BSA standard (5 and 10 µg/µl). The concentration of albumin in the sample were measured by microplate reader at λ 596 nm.

RESULTS

Vero Cell Culture on The Transwell Polyester Permeable Membrane

One of the big difficulty in the culturing cell on the permeable membrane was the determination whether the cell culture have reached confluency without destroy the cells. It risen since the shape of the cell before seeding almost the same with the shape of membrane pores. Recent study used microscope to visualize the cell growth on the membrane. Through the microscope, the membrane pores had perfectly circular shape, while the cells' shape were

not perfectly circular and slight larger than the pores (Fig. 1). By the time the cells grew up and the shape become larger and covered the permeable membrane as well as its pores.

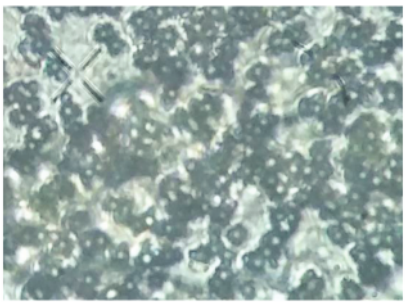

FIGURE 1. Microscopic view of vero cell cultured on the Polyester permeable membrane (24 hours after seeded).

Effect of Dengue Virus Infection on The Characteristic of Vero Cells Permeability

After 2 hours incubated by dengue virus-2, the permeability of vero cell culture increased and caused albumin migration to the lower chamber of the permeable membrane. Table 1 shows the leakage volume of the complete media containing BSA across the cell culture and permeable membrane of dengue virus infected-cells, high titer dengue virus infected-cells and non infected cells (control).

TABLE 1. Measurement of Albumin Leakage Using 5% Standard

Sample	Leakage Volume (µl)	Albumin Content (%)	Albumin Total (µg)	Leakage Capacity (µl/h)	Albumin Clearance (µg/h)
Control 24 h	280 ± 22.91	29.10 ± 1.62	81.50 ± 4.37	11.67 ± 0.67	3.39 ± 0.24
Control 48 h	50 ± 10.22	29.70 ± 1.91	14.85 ± 0.86	2.08 ± 0.13	0.62 ± 0.04
Control 72 h	300 ± 15.27	31.70 ± 1.54	95.10 ± 3.77	12.50 ± 0.55	3.96 ± 0.23
DV-2 24 h	130 ± 15.15	30.71 ± 1.01	39.92 ± 1.98	5.42 ± 0.32	1.66 ± 0.12
DV-2 48 h	370 ± 20.23	30.48 ± 1.36	111.22 ± 5.48	15.42 ± 1.67	4.63 ± 0.36
DV-2 72 h	1080 ± 25.53	31.06 ± 1.84	335.43 ± 21.24	45.00 ± 4.36	13.98 ± 0.92
DV-2 hi-titer 24h	1475 ± 34.12	31.10 ± 1.24	458.79 ± 20.65	61.46 ± 3.17	19.12 ± 1.53
DV-2 hi-titer 48h	1255 ± 25.85	30.79 ± 1.42	386.36 ± 19.32	52.29 ± 4.68	16.10 ± 1.91
DV-2 hi-titer 72h	1450 ± 33.21	31.09 ± 0.94	450.88 ± 22.35	60.42 ± 4.31	18.78 ± 1.35

Transwell containing low titer dengue virus-infected vero cells resulted leakage albumin with volume constantly increased from 24 up to 72 hours. Significant increase occured after 48 hours post infection as shown at Table 1. At the high titer virus, the leakage at the 24h almost ten folds compared to low titer dengue virus. But there is no increase of leakage during 72 hours. Similar with the high titer virus, thereis no increase of albumin leakage along 72 hours, but the value far below the infected cells.

The leakage albumin in the control decreased at 48 h. The same trend also found in the high titer results. Those result was similar to the result found another research [7], where the value of albumin migrates across the endothelial cells had the increase-decrease curve.

DISCUSSION

Vero cells is considered as the standard model cell in dengue studies due to its infectability in response to dengue infection. Previous research found that in response to dengue infection, epithelial cells A549 can release 26 cytokines/chemokines including TNF-α, TNF-β, IL-1 α, IL-1 β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17, G-CSF, GM-CSF, IFN-α2, IFN-β, MCP-1, MIP-1α, MIP-1β and Eutaxin. Those cytokines are responsible for the increase of cell permeability [10].

In relation to previous researchs, recent study has shown the evidence of the increase of permeability when the vero cells were infected by dengue virus. The permeability of the vero cell infected by dengue virus increases by the time. The leakage shown by low titer of dengue virus agrees with the result found by previous research [10], that reported the growth of virus number in the vero cells especially 24 hours post infection. The increase of virus number will increase the cytokines production, which in turn increases the cell permeability and causes the fluid leakage as found in the recent research.

At the samples with high titer of dengue virus, although the albumin leakage tends to stable without any significant increase, the values at the first day almost ten folds compared to low titer samples. A slight reduction found at the second day, but increases again at the third day post infection. The higher value of albumin leakage as found in the vero cells infected by higher titer of dengue virus occurs due to higher number of virus that infect the vero cells and causes the release of higher concentration of cytokines. Such cytokines affect the tight junction of the cells and causes the fluid leakage. Far less leakage was found at the control where the average leakage was about one fifth of the samples with high titer of dengue virus. These results agree with the previous result found by previous researchs [7, 13] where the significant effect of dengue infection including leakage occurs at the second or third days post infection. Recent finding that uses epithelial cells as the target cells for dengue virus also satisfy to the previous research that reported the increase of porcine renal cell line LLC-PK1 when exposed to TNF- α, one of the cytokine that released by epithelial cells in response to dengue infection [14]. The evidence of the increase of the permeability of monkey kidney in response to dengue infection as found in the recent study may help to explain why the mortality of dengue patients with acute kidney injury was higher than those without kidney injury as reported by previous researchs [15, 16, 17].

CONCLUSIONS

The results of the recent research have presented that Dengue virus serotype 2 can infects and causes the leakage of albumin on the culture of vero cell line CCL-81. The intensity of the cell culture leakage highly affected by the titer of the Dengue virus, the higher the titer, the higher the leakage. The result of the recent study may be can be used to explain why the mortality of dengue patients with acute kidney injury was higher than those without kidney injury as found in previous researchs.

REFERENCES

1. S. B. Halstead, *Dengue*, (Imperial College Press, Singapore, 2008), pp. 1-9
2. WHO, *Dengue, guidelines for diagnosis, treatment, prevention and control,* (TDR, France, 2009) pp.3-17
3. A. Srikiatkhacorn , S. Green, Curr. Top Microbiol Immunol, **338**, 67-82 (2010)
4. J. E. Cardier, B. Rivas, E. Romano, AL. Rothman, C. Perez-Perez, M. Ochoa, A.M. Caceres, M. Cardier, M. Guevara, Giovanetti, Endothelium, **13**, 334-340 (2006)
5. C. A. Basilio-de-Oliveira, G.R. Aguiar, M.S. Baldanza, O.M. Barth, W.A. Eyer-Silva and M.V. Paes, Brazilian Journal of Infectious Disease **9(3)**, 341-347 (2005)
6. S. M. Bonner, M.A. O'Sullivan, J. Virol Method, **71(2)**, 159-167 (1998)
7. E. D. Beti, T. Takasaki, I. Kurane, Journal of General Virology, **89**, 842-852 (2008)
8. Y. Yasumura , M. Kawakita, Nippon Rinsho, **21** (6): 1201–1219 (1963).
9. Anderson, S. Wang , Osiowy, Issekutz, 1997, J. Virol, **71**: 4226-4232 (1997).
10. B. Yohan, Indah Kendarsari, K Mutia,2, A. Bowolaksono, Harahap AR., Tedjo Sasmono, *Acta Virologica,* **58**: 20-27 (2014)
11. C. T. Capaldo, A. Nusrat , Biochim Biophys Acta , **1788 (4)**: 864-871 (2009)
12. Casnocha, PhD. Tesis, Rice University (1990)
13. M. Dewi, S. R. Umniyati, Tropical Medicine Journal, **03(02)**, 95-102 (2013)

14. J. M. Mullin, K. V. Snock, Cancer Res, 50: 2172-6 (1990)
15. M. A.M. Khalil, S. Sarwar, M. A. Chaudry, B. Maqbool, Z. Khalil, J. Tan, S. Yaqub and S. A. Hussain, Clin. Kidney J. **5**: 390–394 (2012)
16. P. Vachvanichsanong, E. McNeil, Kidney Involvement in Dengue Viral Infection (Supplement), **46**: 108-117 (2015)
17. M. Kuo, P. Lu, J. Chang, M. Lin, J. Tsai, Y. Chen, Y. Chen, K. Chang, H. Chen, S. Hwang, Clin J Am Soc Nephrol. Sep; **3(5)**: 1350–1356 (2008)

Effect of Dengue Virus Infection on The Permeability of Vero Cells line

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6