A Protein Kinase-A Inhibitor, KT5720, Suppressed Cytopathic Effect Caused by Vesicular Stomatitis Virus

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I investigated the effect of KT5720, an inhibitor of protein kinase A, on the vesicular stomatitis virus (VSV) infection in BHK- 21cell cultures . The virus inducted cytopathic effect (CPE) was almost completely suppressed by KT5720 at 5uM. The inhibitor, however, did not affect replication of the virus nor the synthesis of viral macromolecules. KT5720, did not block the cytoskeletal disruption, while the cell rounding was suppressed. And, the KT5720-sensitive function may be involved in developing the VSV-induced CPE, but not essential for the virus replications.

Key words — Vesicular stomatitis virus (VSV), KT5720, cytopathic effect (CPE)

Introduction

The Nagative-stranded Rhabdovirus genomic RNA encodes at least five species of viral proteins (N, NS, M, G, and L), two of which, L and NS, serve as catalytic and regulatory subunits of the viral RNS polymerase, repectively and are involved in the synthetic process of both viral mRNAs and genomeic RNA [12]. In the case of productive infection of rhabdoviruses, morphological changes of host cells are usually associated with the virus replication, such as the cell rounding, and finally with cell death and cytolysis. Sequential destruction of cytoskeletal structures seems to be related to these morphological changes of the infected cell [25,28]. At least two events have been suggested to be involved in the VSV-induced CPE. One is the inhibition of host macromolecule synthesis occurring in the nucleus (shutoff), and another is the cytoplasmic events which would cause dysfunction of cellular organelles including cytoskeletons and may also cause morphological changes of the cell. As for the former event, at least two viral gene products are suggested to be involved. One is plus-strand leader RNA produced during the course of viral transcription [6,17,31], that are supposed to enter the nucleus and cause the shutoff [14]. Recently, however, the viral matrix (M) protein has also been shown to inhibit the host cell transcription [1]. The M protein is involved essentially in the regulation of viral RNA synthesis [2,4] and virion formation through budding process at the cell membrane in collaboration with the viral glucoprotein (G) and nucleocapsis [9,23]. Its interaction of M protein with both the viral nucleocapsid and G protein have been demonstrated *in vivo* and *in vitro* [5,9,29,32]. From the studies with temperature-sensitive mutants, however, M protein has been thought to display cytotoxicity in the infected animal cells [24]. Recently, the cytotoxicity could be reproduced in the cells by transfecting the M cDNA with a help of expression vector [2].

In my previous studies [13] on the antiviral screening of several protein kinase inhibitors against the VSV replication in cultured animal cells, and I found that KT5720 (protein kinase A inhibitor) suppressed the VSV-induced cytopathic effect without affecting the virus replication and progeny yield [13]. In this report, I further investigated the inhibitory mechanism of KT5720 on the VSV induced CPE.

Materials and Methods

Viruses, cell culture and infectivity assay

The new jersey serotype of VSV (New Jersey serotype) was used throughout this study [10] Stockes of the virus were prepared by infecting to BHK-21 cells at a low m.o.i., and assayed by plaque formation on BHK-21 cell monolayers. Sindbis virus was the same strain described previously [8], and also propaqated and assayed in BHK-21 cell cultures. BHK-21 cell was propagated in Eagle's MEM containing 10% Tryptose phosphate Broth (TPB; Difco Laboratories, Detroit) and 5% calf serum[10, Kyoto University. Akihiko Kawai Lab] and maintained in MEM containing 1% fetal bovine serum[10, Kyoto University.

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Akihiko Kawai Lab]after the virus infection.

Assay of actinomycin D-resistant RNA synthesis.

BHK-21 cell monolayers prepared in 35-mm dishes were infected with VSV at an m.o.i.. of 10 PFU/cell, and fed with fresh medium containing 1% serum. Protein kinase inhibitors were added at the initial step of virus infection at various concentrations as noted in the text. After incubation for 4.5 hr at 37℃, the medium was replaced by the radio-labelling medium which was composed of Eagle' MEM and 5 µg/ml actinomycin D (act. D). After further incubation for 30min, 1-10 µCi/ml [5-^{3}H]uridine was added to the medium, and the culture were incubated for one or two hours. Then, the cultures were washed twice with PBS and lysed by adding one tenth volume of 10% sodium dodecyl sulfate (SDS). After adding 1 mg bovine serum albumin (BSA), the lysates were precipitated with 5% trichloroacetic acid (TCA). The TCA-insoluble materials were recovered onto glass filters (GF/C,2.4-cm; Whatman International Ltd, Maidstone) to determine the radio-activity incorporated into the acid-insoluble fraction by using a liquid scintillation counter (Beckman LSC-70000).

Metabolic labeling of viral proteins with radioactive precursor

Radioactive viral proteins were prepared for studies of autoradiography and immunoprecipitation. Infected cultures were prepared as described above, and fed with fresh medium containing 1% serum. At zero time of incubation, protein kinase inhibitors were added at various concentrations as noted on the text. After incubation for 4.5 hr at 37℃, the medium was replaced by the radio-labelling medium which contained a low concentration (1.5 mg/ml) of cold l-methionine and 1% serum. After further incubation for 30 min, ^{35}s-methionine was added to the culture, which were then incubated for metabolic labeling of infected cells for one or two hourses as noted in the text. The cells were lysed with SDS-sample buffer for SDS poly-acrylamide gel electrophoresis (PAGE).

SDS-PAGE and autoradiographic analysis.

For the electrophoresis of viral proteins in an SDS-PAGE gel, the infected cells were lysed with Laemmli's sample lysis buffer [15] and applied to 10% SDS-PAGE as noted previously [26]. For better sepuratuin of VSV N and proteins in the SDS-PAGE gel, pH of the separation gel was adjusted to 9.5 instead of 8.8. The gels were dried onto Whatmanfilter paper and exposed to an imaging plate for autoradiography. The plate was subjected to the analysis in a BAS-2000 Imaging Analyzer (Fuji Film Co. Ltd., Tokyo).

Antisera

Anti-VSV G antiserum was prepared by immunizing rabbits with the G protein sample which was extracted from SDS-PAGE gels after the electrophoresis of purified VS virions. Anti-VSV NS antiserum was prepared by immunizing rabbits with synthetic oligopeptides which were systhesized by mimicking the amino acid sequence of NS protein.

Immunofluorescence studies

Infected BHK-21 cells were prepared on converslips and fixed at the time indicated in the text with acetone or 3% parafolmaldehyde for 10 to 15min at room temperature (In the case of the paraformaldehyde-fixed specimens, cell membrabe was permeabilized after the fixation by treatment with 1% Triton x-100/PBS). The fixed specimens were first stained with the first antibody against the viral proteins or cytoskeletal components for 60 min at 37℃, and then washed for 1 hr. Then, the specimens were stained with FITC or rhodamine-conjugated second antibody for 30 min at 37℃, and were washed (PBS). The immunostained specimens were observed under a Nikon epi-fluorescence microscope.

Extraction of cellular DNA and agarose gel electrophoresis.

Cellular DNA was extracted according to Gross-Bellard et al. [7]. At various hours of infection, the virus infected and mock-infected cells were recovered with rubber scrapers from the culture vessels, suspended in PBS and sedimented by low speed centrifugation. Then the cells were lysed with 0.1% SDS and treated with proteinase K (100 µg /1 ml) for 90 min at 50℃, and then subjected to phenol/chloroform extraction. The DNA samples obtained were digested with RNase A in 0.1×TE for 30 min at 37℃ and then applied to 1% agarose gel electrophoresis.

Immunoblot analysis

Infected and mock-infected cells were lysed with SDS-lysis buffer. The lysates were applied to 10% SDS-PAGE, and then blotted onto nitrocellulose filter(S &

S). After the blocking procedures, the filter was processed for immunological detection voral proteins with rabbit antisera against the VSV G, M and NS proteins and peroxidase-conjugated anti-rabbit antibody. Color was developed as described previouly [22].

Chemicals and buffers

PBS is composed of 140 mM NaCl, 2.6 mM KCl, 8 mM sodium phosphate, 0.5 mM MgSO and 0.5 mM $CaCl_2$ (pH7.8). PBS(-) means that magnesium and calcium are omitted from PBS. NTE contained 150 mM NaCl, 10 mM Tris-HCl (pH7.8) and 1 mM EDTA. TE contained 10 mM Tris-HCl (pH 7.4) and 1mM EDTA. IP buffer is composed of 0.1%SDS, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate and 10 mM Tris-HCl (pH 7.5). K-252a-derived protein kinase inhibitors, KT5720 and KT5926 (27,28) were purchased from Kyowa Medex Inc. (Tokyo). ^{35}S-methionine or Tran^{35}S-label (specific activity=~1000 Ci/mmol) and [5-^{3}H]uridine (specific activity=~30 Ci/mmol were of ICN Radiochenicals (Irvine, CA).

Results

Effect of K-252a derivatives on the VSV infection in culture

In our previous studied on the antiviral activity of K-252a derivatives against the VSV infection in cell cultures, we found that VSV replication was inhibited by a parent compound (k-252a), that is known to inhibit a broad spectrum of protein kinases. Similar results were also obtained with a myosin light chain kinase inhibitor, KT5926 [13]. Contrasting effect was observed with KT5720, a protein kinase A inhibitor, that suppressed the VSV-induced CPE (cell rounding), but allowed almost a full achievement of VSV replication. The IC_{50} of KT5720 determined on the basis of CPE suppression was about 0.55 uM, while that of KT5926 was 1.5 uM.

Table 1 showes the time course study of VSV-induced CPE and the suppression by the inhibitors. When KT5720 and KT5926 were added to the VSV infected cultures at a concentration of ten times of IC_{50} ,they similarly inhibited the CPE development under the same infection conditions. In the control cultures, the yield of progency virus usually reached the maximum level at around 8 to 10 hr of infection. KT5926 decreased the yield in consistent with suppression of the CPE (15 uM KT5926 reduced the yield

Table 1. Time course study of the effect of KT5720 and KT5926 on the VSV-induced CPE in BHK-21 cell cultures.

Virus infection	Drug addition	Grade of CPE				
		Time p.i. (hr)				
		0	3	6	9	12
-	-	-	-	-	-	-
	KT5720	-	-	-	-	-
	KT5926					
+	-	-	+	++	++~+++	+++~
	KT5720	-	-	-	-	+
	KT5926	-	-	-	-	++~

BHK-21 cell monolayer cultures were infected with VSV at an m.o.i. of 10 p.f.u./cell. After the viral adsorption, maintenance medium was added to the cultures, to which 5 µM KT5720 or 15 µM KT5926 was added immediately. The cultures were incubated at 37°C and the grade of virus-induced CPE was determined under a light microscope at 3-hr interval up to 12 hr after the infection. The grade of CPE is determined according to the cell population showing the cytopathic effect (cell rounding and/or cytolysis): -, negative; +, 10-25%; ++, 50%; +++, 70-80%; ++++, 100%.

by 90% or more even at 12 hr), while 5 uM KT5720 only slightly inhibited the virus replication, and the yield reached the maximum level at around 12 hr, Similar effect was observed with these inhibitors in the viral RNA synthesis (data not shown). Fig. 1. compares the dose-response curves: KT5926 decreased the viral RNA synthesis, while almost a maximum level of viral RNA synthesis was observed in the presence of 5 uM KT5720, that strongly suppressed the VSV-induced CPE (Table 1). Effect of KT5720 was further investigated here as follows (Detailed studies of the inhibitory effect of KT5926 on the VSV replication will be described elsewhere).

Effect of VSV infection on the integrity of the host cell DNA

Before starting further studies on the mechanism of CPE suppression by KT5720, I checked whether the cellular DNA is fragmented during the course of CPE development and whether such event could be blocked by KT5720. As shown in Fig. 2A, VSV infection did not seem to cause fragmentation of cellular DNA in BHK-21 cells even when the virus-induced cell rounding reached the maximum level and cytolysis began to occur at 24 to 36 hr. And, the inhibitor (KT5720) did not change the results of experiments on the cellular DNA (Fig. 2A). In another experiment performed in parallel with Sindbis virus (Fig. 2B), we detected
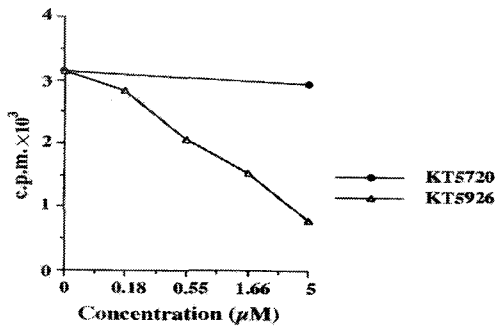
Fig. 1. Dose-response curve of the effect of KT5720 and KT5926 on the viral RNA synthesis. Various amount of drugs were added to the cultures, that were then incubated for 6 hr at 37℃. Actinomycin D (5 μg/ml) and [5-³H]uridine (1 μCi/ml) were added to the cultures at 1.5 and 2 hr after the infection, respectively. The cells were recovered and lysed with 1% SDS and then treated with 5% TCA on ice to determine the radioactivity incorporated into the acid-insoluble fraction as described under materials and methods. Symbols: ●, KT5270; △, KT5926
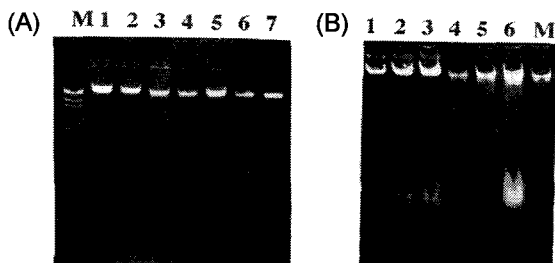

Fig. 2. Agarose gel electrophoretic analysis of host cell DNA from the VSV and Sindbis virus infected cells. BHK-21 monolayers in 60 mm dishes were infected with VSV (A) or Sindbis virus (B) at an m.o.i. of p.f.u./cell, and incubated at 37℃ for up to 24 to 36 hr, the time when the virus-induced cell rounding reached the maximum level and the cytolysis began to occur. Mock-infected cultures were also prepared in parallel. At 6 or 12 hr intervals (VSV infection) or at 24 hr (Sindbis virus infection), cells were recovered and subjected to DNA extraction as described under materials and mathods. In the case of VSV, DNA extracted from a 60-mm dish was dissolved in 500 μl 0.1×TE, from which 15 μl (8 μg DNA) was taken and applied to the agarose gel. In the case of Sindbis virus infection, the DNA was dissolved in 500 μl 0.1×TE, from 2 and 3: DNA from which 5, 10 and 15 μl were taken out and applied to the gel. (A) Lanes 1, 2 and 3: DNA from uninfected control (obtained at 6, 24 and 36 hr, respectively); lanes 4, 5, 6, and 7: DNA from uninfected (6, 16, 24, 36 hr, respectively). (B) Lanes 1, 2 and 3: DNA from uninfected control cells (2.7 μg, 5.3 μg and 8 μg DNA, respectively); lanes 4, 5 and 6: DNA Sindbis virus infected cells (1.3, 2.7, 4 μg DNA, respectively). Lane M : size marker (HindⅢ-digested λ phage DNA).

the DNA fragmentation in the infected cells as reported by Levine et al. [16]. In addition, we also observed the formation of blebs and apoptotic bodies in the Sindbis virus infected cultures, while such morpholigical changes were not observed in the VSV infected cultures (data not shown). These results suggest that the VSV infected cells are killed by a non-apoptotic process.

Studies on the effect of KT5720 on the viral protein synthesis

In order to investigate the mechanism of CPE suppression by KT5720, we next examined whether the inhibitor affected the viral protein synthesis, post-translational processing or the intracellular behaviour of the viral protein Effect on viral protein synthesis was first investigated as follows.

As shown in Fig 3, almost the same migration patterns were observed in SDS-PAGE of the viral proteins produced in the presence and absence of KT5720. This figure also shows that KT5720 did not affect the VSV-induced shutoff of host protein synthesis. Similar results were also obtained when treated from the earlier phase of infection (data not shown). Immunoblot analysis with anti-G and anti-NS antisera did not show any difference between the viral proteins produced in the KT5720-treated and mock-treated infected cells (Fig. 4). Semi-Quantitative immunoblot assay indicated that the rate of viral protein synthesis
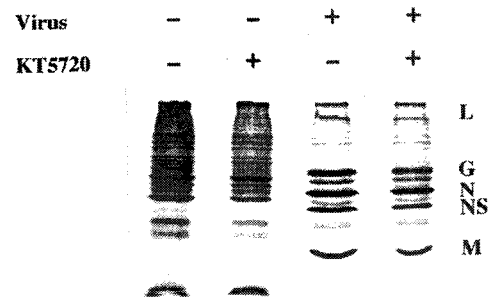

Fig. 3. SDS-PAGE and autoradiographic analysis of the effect of KT5720 on the viral protein synthesis. VSV infected and uninfected BHK-21 cells were mock-treated with 5 μM KT5720 from 4.5 hr of infection, and labeled with ³⁵s-methionine for one hour (from 5th to 6th hour of infection). The cells were then washed with PBS and recovered by lysing with a sample lysis buffer. After electrophoresis a in SDS-PAGE gel (pH 9.5), the gel was subjected to autoradiography (see materials and methods). Lane 1: mock-treated control; lane 2: KT5720-treated control; lane 3: VSV infected and mock-treated; lane 4: VSV infected and KT5720-treated.

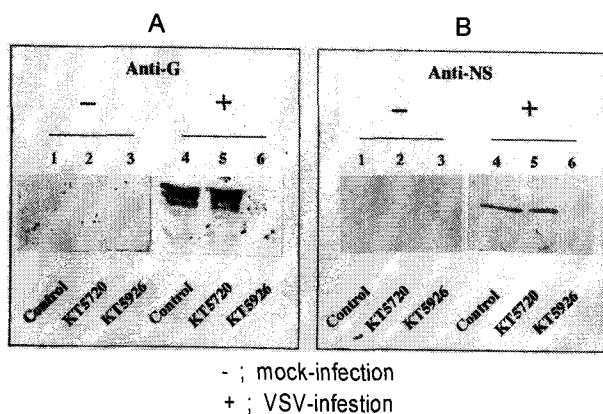- ; mock-infection
+ ; VSV-infestion

Fig. 4. Immunoblot analysis of viral protein synthesis in the KT5720 and KT5926-treated cultures. VSV infected mock infected cells were prepared as described for Fig. 1, and incubated in the presence or absence of either 5 μM KT5720 or 15 μM KT5926 for 5 hr at 37℃. Then, the cells were recovered with rubber srapers, and lysed with a sample lysis buffer. The lysates were subjected SDS-PAGE and immunoblot analysis with rabbit antibodies against the viral G, NS and M proteins as described under materials and methods (A and B). Immunoblots stained with anti-G and anti-NS antibodies, respectively: lane 1, uninfected untreated control; lane 2, KT5720-treated control; lane 3; KT5926-treated control; lane 4, VSV infected untreated control; lane 5, VSV infected and KT5720-treated; lane 6, VSV infected and KT5926-treated.

did not seem to be altered by KT5720. These results suggest that viral protein systhesis was not affected by KT5720, in other words, the KT5720-sensitive functions are not involved in the viral protein synthesis.

Studied on the distribution of viral antigens in the cell

We next performed immunofluorescence studies on the viral protein synthesis. Fig. 5. shows the time course studies on the synthesis and distribution of the viral glycoprotein (G) in the KT5720-treated and mock-treated cells. Most cells become antigen-positive at around 6 hr, and morphological changes began to appear at around 6 hr in the mock-treated culture. On the other hand, most viral antigen-positive cells preserved the flat shape even at 12 hr, at which most infected cells displayed rounding in the mock-treated culture.

Most of these antigen-positive cells still displayed a moral flat morphology even at control cultures (Fig. 5). KT5720 did not cause any changes in the uninfected cultures for at least 12 hr of incubation. These observations indicate that
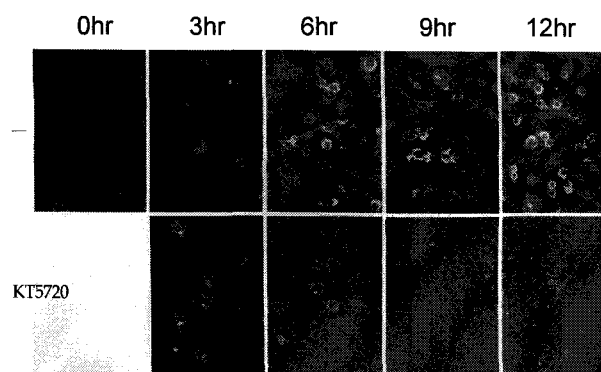

Fig. 5. Immunofluorescence study of the effect of KT5720 on the morphology of viral antigen-positive cells. VSV infected BHK-21 cells sown on coverslips were incubated in the presence and absence of 5 μM KT5720 for 12 hr. At each 3-hr interval, the cells were fixed with acetone for 15 min at room temperature. Acetone-fixed samples were stained with rabbit anti-G antibody (first antibody) and then with rhodamine-conjugated second antibody. Photos: (left column) mock-treated infected cultures at 0, 3, 6, 9 and 12 hr (from top to bottom); (right column) KT5720-treated infected cultures at 3, 6, 9, and 12 hr (from top to bottom).

KT 5720 suppressed the VSV-induced cell rounding without affecting the distribution of M protein. When 5 uM KT5720 was added to the infected cultures, the morphological change (cell rounding) was abolished (Fig. 5). In other words, the inhibitor did not block the VSV-induced disruption of microtubules and intermediate filaments.

Discussion

In this study, I investigated the effect of a protein kinase A inhibitor, KT5720, on VSV infection in cell cultures. The inhibitor suppressed the VSV- induced cell rounding without affecting the viral gene expression as well as progeny virus production. In other words, the progeny virus production can be achieved without causing the rounding of host cells which is usually associated with the virus replication, and the cell rounding may depend on the KT5720-sensitive function(s). Since the virus-induced shutoff of cellular protein synthesis was not affected by KT5720 (Fig. 3) it was reconfirmed that the VSV-induced shutoff and cell rounding are independent events, although both depend on the viral gene expression. At present, I think that the KT5720-sensitive function(s) involved in the VSV-induced cell rounding is a cellular function(s), because our recent study suggests that the L protein-asso-

ciated protein kinase activity is not sensitive to KT5720, and other viral proteins are throught to have no enzymatic activity.

Rounding of the cell may be caused by many kinds of agents and biological products which affect the cytoskeletal structures, such as microtubule-destroying agents (e.g., Vinblastine), virus infections, Simon, K.O. et al. [28] described sequential disassembly of three cytoskeletal components (i.e., microfilament, microtubule and intermediate filament) in the VSV infected cells. Melki et al. [18] reported recently that the VSV-induced cell rounding may be reflection of the disruption of microtubule networks, which might be caused by intersction between the viral M protein and tubulin.

Our present study showed that KT5720 did not affect the interaction between the viral M protein and cytoskeletal components (data not shown), however, the agent suppressed the cell rounding. Concerning this problem, I would like reconsider the recent studies performed in the Kawai's laboratory (Kyoto) on the functional interaction of rabies virus proteins with cellular or cytoskeletal components which may be involved in the viral replicative process. They have assumed recently that some of such components may also be incorporated into mature virions as have been reported for the action in the virion [20].

Acknowledgement

References

1. Black, B. L. and D. Lyles. 1992. Vesicular stomatitis virus matrix protein inhibits host cell-directed transcription of target genes in vivo. J. Virol. 66, 4058-4064.

2. Blondel, D., G. G. Harmison and M. Schubert. 1990. Role of matrix protein in cytopathogenesis of vesicular stomatitis virus. J. Virol. 64, 1716-1725.

3. Carroll, A. R. and R. R. Wagner. 1979. Role of the membrane (M) protein in endogenous inhibition of in vitro transcription by vesicular stomatitis virus. J. Virol. 29, 134-142.

4. Clinton, G. M., S. P. Little, F. S. Hagen and A. S. Huang. 1978. The matrix (M) protein of vesicular stomatitis virus regulates transcription. Cell 15, 1455-1462.

5. Chong, L. D. and J. K. Rose. 1993. Membrane association of functional vesicular stomatitis virus matrix protein in

6. Grinnell, B. W. and R. R. Wagner. 1983. Comparative inhibition of cellular transcription by vesicular stomatitis virus serotypes New Jersey and Indiana: role of each viral leader RNA. J. Virol. 48, 88-101.

7. Gross-Bellard, M., P. Oudet and P. Chambon. 1973. Isolation of High-Molecular-Weight DNA from Mammalian Cells. J. Biochem. 36, 32-38.

8. Honda, Y., A. Kawai and J. Matsumoto. 1984. Comparative Studies of Rabies and Sindbis Virus Replication in Human Neuroblastoma (SYM-I) Cells that Can Produce Interferon J. Gen. Virol. 65, 1645-1653.

9. Kaptur, P. E., R. B. Rhodes and D. S. Lyles. 1991. Sequences of the vesicular stomatitis virus matrix protein involved in binding to nucleocapsids. J. Virol. 65, 1057-1065.

10. Kawai, A. 1977. Transcriptase Activity Associated with Rabies Virion. J. Virol. 24, 826-835.

11. Kase, H., K. Iwahashi, S. Nakanisji, Y. Matsuda, K. Yamada, M. Takahashi, C. Murakata, A. Sato and M. Kaneko. 1987. K-252 compounds, novel and potent inhibitors of protein kinase C and cyclic nucleotide-dependent protein kinases. Biochem. Biophys. Res. Comm. 142, 436-440.

12. Kim, Y. S. and A. Kawai. 1998. Studies on the AntiviralMechanisms of protein kinase inhibitors K-252a and KT5926 against the replication of Vesicular stomatitis virus. Biol. Pharm. Bull. 21, 498-505.

13. Kim, Y. S., J. Sagara and A. Kawai. 1995. Studies on the Antiviral Activity of protein kinase inhibitors against the replication of Vesicular stomatitis virus. Biol. Pharm. Bull. 18, 895-899.

14. Kurilla, M. G., H. Piwnica-Worms and J. D. Keene. 1982. Rapid and Transient Localization of the Leader RNA of Vesicular Stomatitis Virus in the Nuclei of Infected Cells. Proc. Natl. Acid. Sci. USA 79, 5240-5244.

15. Laemmli, U. K. 1970. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature London 227, 680-685.

16. Levine, B., Q. Huang, J. T. Isaacs, J. C. Reed, D. E. Griffin and J. M. Hardwick. 1993. Conversion of lytic to persistent alphavirus infection by the bcl-2 cellular oncogene. Nature 361, 739-742.

17. McGowan, J. J., S. U. Emerson and R. R. Wagner. 1982. The plus-strand leader RNA of VSV inhibits DNA-dependent transcription of adenovirus and SV40 genes in a soluble whole-cell extract. Cell 28, 325-333.

18. Melki, R., Y. Gardina and D. Blondel. 1994. Interaction between tubulin and the viral matrix protein of vesicular stomatitis virus: possible implications in the viral cytopathic effect. Virology 202, 339-347.

19. Morimoto, K., Y. J. Ni and A. Kawai. 1992. Syncytium formation is induced in the murine neuroblastoma cell cultures which produce pathogenic type G proteins of the rabies virus. Virology 189, 203-216.

20. Naito, S. and S. Matsumoto. 1978. Identification of cellular actin within the rabies virus. Virology 91, 151-163.

vivo. J. Virol. 67, 407-414.

21. Nakanishi, S., K. Yamada, H. Kase, S. Nakamura and Y. Nonomura. K-252a, a novel microbial product, inhibits smooth muscle myosin light chain kinase *J. Biol. Chem.* **263**, 6215-6219.

22. Nakanishi, S., K. Yamada, K. Iwahashi, K. Kuroda and H. Kase. 1990. KT5926, a Potent and Selective Inhibitor of Myosin Light Chain Kinase. *Molec. Phamacol.* **37**, 482-488.

23. Newcomb, W. W. and J. C. Brown. 1981. Role of the vesicular stomatitis virus matrix protein in maintaining the viral nucleocapsid in the condensed form found in native virions. *J. Virol.* **39**, 295-299.

24. Ono, K., M. E. Dubois-Dalcq, M. Schubert and R. A. Lazzarini. 1987. A mutated membrane protein of vesicular stomatitis virus has an abnormal distribution within the infected cell and causes defective budding. *J. Virol.* **61**, 1332-1341.

25. Rutter, G. and K. Mannweiler. 1977. Alterations of Actin-Containing Structures in BHK21 Cells Infected with Newcastle Disease Virus and Vesicular Stomatitis Virus. *J. Gen. Virol.* **37**, 233-242.

26. Sagara, J. and A. Kawai. 1992. Identification of heat shock protein 70 in the rabies virion. *Virology* **190**, 845-848.

27. Sagra, J., S. Tsukita, S. Yonemura, S. Tsukita and A. Kawai. 1995. Cellular actin-binding ezrin-radixin-moesin (ERM) family proteins are incorporated into the rabies virions and closely associated with viral envelope proteins in the cell. *Virology* **206**, 485-494.

28. Simon, K. O., P. A. Whiitaker-Dowling, J. S. Youngner and C. C. Widnell. 1990. Cited by other Osatomi and Sumiyoshi, Complete Nucleotide Sequence of Dengue Type 3 Virus Genome. *Virology* **177**, 289-305.

29. Sun, Y. W., K. Suryanarayana, P. Jusjtice, D. Robinson and R. R. Wagner. 1994. Membrane-binding domains and cytopathogenesis of the matrix protein of vesicular stomatitis virus. *J. Virol.* **68**, 7386-7396.

30. Tsukita, S., K. Oishi, N. Sato, J. Sagara, A. Kawai and S. Tsukita. 1994. ERM Family Members as Molecular Linkers between the Cell Surface Glycoprotdn CD44 and Actin-based Cytoskeletons. *J. Cell Biol.* **126**, 391-401.

31. Weck, P. K., A. R. Caeeol, D. M. Shattuck and R. R. Wagner. 1976. Use of UV Irradiation to Identify the Genetic Information of Vesicular Stomatitis Virus Responsible for Shutting Off Cellular RNA Synthesis. *J. Virol.* **30**, 746-753.

32. Willson, T. and J. Lenard. 1981. Interaction of wild-type and mutant M protein of vesicular stomatitis virus with nucleocapsids in vitro. *Biochemistry* **20**, 1349-1354.

초록 : Protein Kinase Inhibitor, KT5720의 VSV에 의한 세포변성 억제 연구

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Nocardiopis sp. 유래의 protein kinase 저해제인 KT5720, KT5926을 사용하여 Vesicular Stomatitis Virus (VSV) 증식에 미치는 세포변성의 억제를 지표로서 연구하였다. 그 결과 cAMP 의존 kinase를 저해하는 KT5720, mysion light chain kinase (MLCK)를 저해하는 KT5926이 VSV 에 의한 세포변성을 억제시켰다.

Protein kinase inhibitor KT5720은 VSV의 증식에 영향을 미치지는 않지만, 세포변성과정은 억제하는 것으로 나타났다. Virus RNA 및 단백질 합성에서 PKA KT5720이 영향을 미치지 않는 것은 virus 증식에 영향이 없는 것으로 나타난다. Virus 증식과정에서 성질이 다른 protein kinase가 관여하는데, 이것은 negative strand virus에 대한 항virus 제 개발에 중요한 역할을 한다고 생각한다.