Suppressive effects of a cyanine dye against herpes simplex virus (HSV)-1 infection

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ABSTRACT

In this study, we demonstrate that a cyanine dye, lumin, significantly suppressed cytopathic effect by herpes simplex virus (HSV)-1 toward human amnionic FL cell and also it reduced replication of HSV-1 in a dose-dependent manner. In addition, lumin additively augmented the antiviral effect of interferon (IFN)-α. Furthermore, fluorescence microscopic study showed that lumin (not IFN-α) itself remarkably induced alkalinization of intracellular organelle, suggesting the inhibition of virus invasion into the cells. These results suggest that lumin exerts an antiviral action against HSV-1 with the independent pathways of IFN-α and also it would become a therapeutically effective drug in clinical practice.

A wide variety of dyestuff has been investigated as molecular probes for specific demonstration of cellular components in the morphological study, the examination of the shape at the ultrastructural level, and the interactions with the other molecules (13). Furthermore, some dyestuff, especially photosensitizing dye has been utilized in the treatment for various types of cancer, age-related muscular degeneration, infectious diseases as well as in diagnosis (1). A cyanine photosensitizing dye, lumin has been well known to show some beneficial effects for human and been used as an oral drug to prophylactic allergy and to promote wound healing. In addition, it has also been shown to enhance the antitumor effects (7), cell-activating effects (8), interferon (IFN)-γ production (5) and Fc-receptor-mediated phagocytic activity of macrophages (15). Herpes simplex virus (HSV)-1 is a nuclear replicating enveloped virus and one of the common infectious viruses in human (2). The HSV-1 infections are distributed worldwide and cause wide range of diseases from mild to severe. In the most cases, the virus lies concealed in ganglions for the lifetime, repeats the cycle of replapses and remissions, also occasionally elicits severe and sometimes fatal diseases in immunocompromised individuals (12). In order to circumvent the problems, development of alternative prophylactic or antiviral agents with different mechanisms of action has been desired for the treatment of HSV-1 recurrence. In the present study, we report favorable antiviral effects of lumin against HSV-1 infection in vitro assay system.

A human amnionic FL cell line was grown in EMEM (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 5% fetal bovine serum (FBS; Gibco BRL; Grand Island Biological Co., Grand Island, NY) under the standard conditions in a humidified 5% CO₂ and air mixture at 37°C. HSV-1 (Miyama strain) was kindly provided from Dr. Masao Yamada of Okayama University. Lumin (4, 4″-(3-[2-(1-ethyl-4(1-H)-quinolinylidene) ethyldene] propenylene) bis (1-ethylquinolinium iodide), MW:789.53) produced by Hayashibara Biochemical Laboratories Inc. was dissolved in dimethylsulfoxide (Sigma-Aldrich Ltd., St. Louis, MO) at the concentration of 5 mg/mL. Natural human IFN-α was purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan). Acridine orange dye was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).
Multiplication of HSV-1 in FL cells was examined with two-step infection and replication of the virus. FL cells (4 × 10^5/well) were seeded in 12-well culture plates and the cells were infected with 50 plaque forming unit (PFU) of HSV-1 in 100 μL of EMEM supplemented with 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid at 37°C for 1 h. After washing with Dulbecco’s phosphate-buffered saline (D-PBS; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), the cells were incubated in 0.5 mL of EMEM with various concentrations of lumin for 2 days. Then the supernatants were recovered and transferred to the independently cultured FL cells in 12-well culture plates at 37°C for 1 h. One mL of 1% (W/V) noble agar (Beckton Dickinson and Company, NJ)-containing EMEM supplemented with 5% FBS was added into respective wells. After incubation for 3 days, the cells were stained with 1 mL of 0.01% (W/V) neutral red (Wako Pure Chemical Industries, Ltd.) solution containing 1% noble agar in the EMEM supplemented with 2% FBS and then the number of plaques was counted. As shown in Fig. 1, the number of plaques was significantly reduced even in a lower concentration of lumin (1 μg/mL, P < 0.05). This result implies that HSV-1 infectivity was suppressed in a dose-dependent manner when the FL cells were exposed to lumin.

Since the type I IFN (IFN-α) is an intrinsic antiviral agent, we next examined the action of lumin in combination with IFN-α. FL cells (2 × 10^5/well) in 96-well plates were cultured at 37°C for 2 days. Then, the cells were incubated with various concentrations of IFN-α and/or 1.3 μg/mL of lumin. After 16 h, the cells were infected with HSV-1 (5000 PFU). After 2 days of culture, viable cells were stained with 0.02% neutral red solution and they were lysed in 30% ethanol containing 7 mM HCl, and then the optical density (OD) at 560 nm of the solution was examined.

As shown in Fig. 2, lumin itself (1.3 μg/mL) showed certain degree of antiviral effect and approximately 40% of cell viability. The antiviral level of lumin plus IFN-α showed additive effects but not synergistic action compared with that of lumin or IFN-α only. It is also well known that when the cells are infected with certain viruses, type I IFNs are secreted by most of cells and bind to IFN-α receptor (IFNAR)-1 resulting in autocrine/paracrine activation of signaling via IFN receptors (3). Although we examined the IFN activity to test the

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**Fig. 1** Effect of lumin on plaque formation of HSV-1 in FL cells. Lumin remarkably reduced the number of plaques in a dose-dependent manner. Results represent one typical experiment of two similar independent experiments (n = 4). Significant levels were determined using Student’s t-test versus control groups, *P < 0.05.

**Fig. 2** Effect of lumin on antiviral activity of IFN-α in FL cells. Lumin showed additive antiviral effect against HSV-1. ( ); IFN-α only, ( ); lumin + IFN-α Results represent one typical experiment of two similar experiments.

**Fig. 3** Effect of lumin against HSV-1 using cytopathic effect assay. Lumin had no direct inhibition effect against HSV-1. ( ); lumin only, ( ); HSV-1 pre-incubated with lumin for 1 h, ( ); co-incubation of HSV-1 and lumin). Results represent one typical experiment of two similar experiments (n = 3).
Suppressive effect of lumin against HSV-1 infection

Suppressive effect of lumin against HSV-1 infection. Acridine orange stains acidic intracellular organelles and the color changes from orange to faint yellow or green (6). FL cells (5 × 10^4/mL) were treated with IFN-α (1,000 IU/mL) or lumin (1 or 3 μg/mL) at ambient temperature for 1.5 h. The cells were seeded and after 24 h, acridine orange solution (1 μg/mL) was added and cultured at 37°C for 15 min. Subsequently, they were washed with D-PBS and visualized by microscope (magnification ×400, Nikon Optiphot, Tokyo, Japan).

As shown in Fig. 4A and 4B, untreated and IFN-α treated FL cells exhibited a bright orange fluorescence, indicating a condition of acidic pH in the perinuclear organelles of cells. It implies that IFN-α has no effects on pH of the endosomes. On the other hand, the orange fluorescence of intracellular vesicles in the lumin-treated cells markedly reduced and granular fluorescence was faintly observed. The density of granular fluorescence of the FL cells treated with lumin (3 μg/mL) relatively decreased in comparison with that of the cells treated at the concentration of 1 μg/mL (Fig. 4C and 4D). When the supernatants were subjected to the plaque assay, the number of plaques dose-dependently decreased [44.0 ± 0.0 (vehicle), 14.3 ± 1.53 (1 μg/mL) and 0 (3 μg/

Fig. 4 Effect of lumin on endosomal pH. The alkalinization of endosomes was induced by lumin but not IFN-α. A: vehicle, B: IFN-α 1000 IU/mL, C: lumin (1 μg/mL), D: lumin (3 μg/mL)
These results propose that alkalinization of endosomes was dose-dependently induced in the cells by the treatment with lumin and the pH changes correlated with the plaque numbers. Intracellular endosomes are maintained at a specific acidic pH value by vacuolar H\(^+\)-ATPase in combination with the other ion transporters and channels (4). Lumin might inhibit the enzyme responsible for the acidification of endosomes and prevent the proton gradient. It has been reported that conformation of an oligopeptide corresponding to DNA polymerase of HSV-1 changes pH-dependently (11). Since the oligopeptide from acidic environment is thought to be important for membrane translocation, the structural changes of the peptides induced by lumin would cause the inhibition of HSV-1 infection into FL cells.

In summary, we demonstrate here for the first time that lumin exhibits suppressive effects against HSV-1 through a different mechanism with that of type I IFNs. In addition, it is not the direct effect for the viral replication but the indirect virus suppression as a result of their cell-mediated reactions. These results suggest that lumin is a potential new preventive anti-herpetic drug together with IFN or the other antiviral drugs against infectious diseases. Further studies would be required to understand the efficacy and mechanism of the action using animal models in future.

REFERENCES