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Photoactivated Pheophorbide a from Silkworm Excreta Causes Irreversible Damages to a Human Tumor Cell and Vescular Stomatitis Virus

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We isolated pheophorbide a from silkworm excreta (SPba) and demonstrated that its significance as a photosensitizer in experimental photodynamic therapy (PDT). In this, we characterized the cell death pathway in tumor cells treated with SPba following the light irradiation. Effect of SPba/PDT on infectivity of vescular stomatitis virus (VSV) was also examined to compare its effect in microbes which lack apoptotic process.

As target tumor cells, a human lymphoid tumor cell line, Jurkat, was chosen. In 30~60 min after photodynamic therapy (PDT) with SPba, apoptotic features such as membrane blebbing and DNA fragmentation were prominent. Rapid release of cytochrome c from mitochondria into the cytosol, activation of caspase 3, and the cleavage of poly ADP-ribose polymerase (PARP) were concurrently demonstrated. However, activation of caspase 8 was not detected throughout the experiments. The self-sufficiency of caspase 3 in the apoptosis of the SPba treated cells was anticipated by results of caspase inhibition experiments. Mitochondria were implicated as the central control point for the apoptosis by confirming SPba specific emission spectra in the mitochondrial fraction and changes in its membrane potential following the light irradiation. Taken together, these results suggest that the mode of Jurkat cell death by SPba/PDT is a mitochondrial apoptosis mediated by caspase-3-pathways triggered by cytochrome c release from mitochondria.

An enveloped animal virus, vesicular stomatitis virus (VSV), was used as a target organism for photodynamic antimicrobial chemotherapy (PACT). For SPba mediated PACT, the viruses in suspensions were treated with varied doses of SPba (15~60 μg/ml) and visible red light fixed to be 120 mJ/cm^2. The antiviral effect of the CpD-PACT was measured in 1 hr after the light irradiation by the extent of suppressions in plaque forming unit (PFU). In cultures inoculated with PACT-treated VSV, suppression in plaque forming unit (PFU) was prominent and the results were demonstrated in a dose-dependent manner. In assays of RT-PCR, a single dose of 30 μg/ml CpD and light caused a complete inhibition of viral RNA synthesis in the host cells,

which agreed with the complete loss of plaque forming activity observed in PFU assays. An *in vitro* transcription assay for viral RNA using ^{3}H-UTP and gel electrophoresis for the level of M protein were conducted. A gradual decrease in viral RNA transcription and an immediate decrease in M protein levels were observed in cells inoculated with the CpD-PACT-treated virus. These results demonstrated that CpD is a potential photodynamic antiviral agent, which causes inactivation of the matrix protein as well as transcription mechanisms involved in the VSV replication.