



Title	Photosensitizer, pheophorbide , induces caspase-3-dependent apoptosis in Chinese hamster V79 fibroblast cells
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Citation	Japanese Journal of Veterinary Research, 47(1-2), 94-95
Issue Date	1999-08-31
Doc URL	<a href="http://hdl.handle.net/2115/2779">http://hdl.handle.net/2115/2779</a>
Type	bulletin (article)
File Information	KJ00003408111.pdf



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plus- or minus- strand hantavirus S genome RNA in which the sensitivities of NB and SS-PCR were 107 and 103 or more viral RNA molecules, respectively.

2. In Vero E6 cells infected with KI-83-262 (KI) strain of Seoul type hantavirus, plus-stranded RNA of the S genome began to synthesize immediately after inoculation followed by minus-strand RNA synthesis, and then infectious virus particles were released into the media from 24 hours after inoculation.

3. Newborn rats were inoculated with KI strain through three different inoculation routes [subcutaneous (s.c.), intranasal (i.n.), per os (p.o.)]. Only s.c. inoculated rats exhibited both plus- and minus-strand virus genome RNA. It was re-

vealed that the s.c. route was an efficient way to induce infection for viral RNA replication in rats.

4. In the s.c. inoculated rats, both plus-strand and minus-strand virus RNA was detected at 1 week after infection, but, only plus-strand virus RNA was detected at 1 month after infection. This indicates that plus-stranded RNA may be predominant in persistently infected animals.

5. Hantavirus genome RNA was detected by SS-PCR not only in s.c. rats but also in i.n. and p.o. rats at 1 week and 1 month after infection. Therefore, even in rats infected through less efficient routes, once the infection is established, extremely small amounts of hantavirus RNA can be persistently sustained.

Photosensitizer, pheophorbide *a*, induces caspase-3-dependent apoptosis in Chinese hamster V79 fibroblast cells.

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Pheophorbide *a* (PPa) is a haematoporphyrin derivative, which is a causal substance of dietary photosensitization in animals. The illumination of haematoporphyrin derivatives such as PPa with visible light is known to produce singlet oxygen ( $^1O_2$ ) and to induce cell death, which is called photodynamic effects. Therefore, haematoporphyrin derivatives are sometimes utilized for the tumor therapy as a photosensitizer. Recently, it was reported that the apoptotic pathway caused a considerable part of photodynamic-induced cell death. Using Chinese hamster V79 fibroblast cells treated with PPa and visible light, the present study was carried out to clarify whether the apoptotic signaling pathway from the release of cytochrome *c* (Apaf-2) from mitochon-

dria to the activation of caspase-9 (Apaf-3) and caspase-3 (CPP32) was involved in photodynamic-induced cell death.

Flow cytometric analysis combined with DNA end-labeling technique using FITC-conjugated-dUTP (TUNEL method) showed that apoptotic cell was induced after exposure of Chinese hamster V79 cells to visible red light (580~700nm) in the presence of PPa and the number of apoptotic cells time-dependently increased. The induction of apoptosis was further confirmed by the appearance of ladder-like DNA fragmentation after the PPa-treatment. Western blot analysis of S-100 fraction of V79 cells revealed that cytochrome *c* (Apaf-2) was released from mitochondria by the PPa-treatment.

Either protease inhibitor, Ac-DEVD-CHO (caspase-3 inhibitor) or Ac-IETD-CHO (caspase-9 inhibitor), significantly attenuated PPa-induced apoptosis. These results, therefore, indicated that the photodynamic treatment caused mitochondria to release cytochrome c (Apaf-2) and the released cytochrome c (Apaf-2) resulted in the activation of caspase-9 (Apaf-3) and caspase-3 (CCP32) followed by the DNA fragmentation. An intracellular  $\text{Ca}^{2+}$  chelator, BAPTA (1, 2-bis *o*-aminophenoxy ethane *N,N,N',N'*-tetracetic acid), and an agent to increase the

concentration of intracellular cAMP, forskolin, showed the ability to inhibit the PPa-induced apoptosis. However, while BAPTA suppressed the release of cytochrome c from mitochondria, forskolin did not, proving that the intracellular  $\text{Ca}^{2+}$  and cAMP independently serve as regulators for photodynamic-induced apoptosis at the upstream of caspases.

These results will provide useful information about the mechanisms of photosensitization by PPa and the application of photodynamic treatments to tumor therapy.

### Lipid peroxides and antioxidants in sera of neonatal hotbred and coldbred horses

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Reactive oxygen intermediates (ROIs) are produced in biological systems such as mitochondria, neutrophils and metabolic processes of toxic substances. Since ROIs induce cell damage by reacting with biological important molecules like lipids, proteins and DNA, various antioxidative substances, superoxide dismutase (SOD), catalase, glutathion peroxidase, bilirubin, vitamin E, ascorbate and uric acid, are present to counteract the harmful reactivity of ROIs. Therefore, it was widely recognized that the imbalance between the ROI production and the antioxidant activity was a cause of various diseases like pulmonary emphysema, diabetes, arteriosclerosis and ischemia-induced injury. Excessive exercise is also inferred to increase ROIs by burst of oxygen consumption. Our previous study showed that the antioxidative activity ( $\text{O}_2^{\cdot-}$ -scavenging activity) of hotbred serum was higher than that of coldbred one, suggesting that

there was a relationship between exercise and oxidative stress in horses. However, it was also found that the amount of lipid peroxides in hotbred serum was significantly higher than that in coldbred one. The present study was carried out to clarify whether this conflicting observation was congenital in hotbred. For this purpose, the age-dependent changes in the amount of lipid peroxides in hotbred serum and its  $\text{O}_2^{\cdot-}$ -scavenging activity were compared with those in coldbred one. The results demonstrated that not only the amount of lipid peroxides but also the  $\text{O}_2^{\cdot-}$ -scavenging activity were found to be significantly higher in serum of neonatal hotbred than in neonatal coldbred, suggesting that the higher amount of lipid peroxides and the higher antioxidative activity in hotbred serum were congenital. The higher antioxidative activity in hotbred serum appears to be explained by the fact that the amounts of ceruloplasmin and ascorbate were