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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

higher in hotbred serum than in coldbred one.

When the amount of lipid peroxides in neonate serum was compared with that of adult one, it was higher in the neonate serum regardless of the difference in breed, indicating that the oxidative stress was stronger in neonate than in adult. However, it was hard to explain this phenomenon by the difference in the amount of

SOD and the  $O_2^{\cdot-}$ -scavenging activity, because no difference was observed between their sera.

These results showing the age-dependent differences in the  $O_2^{\cdot-}$ -scavenging activity and the amounts of antioxidative substances between hotbred and coldbred horses will provide useful information for susceptibility of horses to exercise-induced oxidative stress.

### Effects of hypoxia on p53-dependent apoptosis and the arrest of cell-cycle progression in X-irradiated human EB-B cells

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Ionizing radiation induces less efficiently cell death under the hypoxic conditions than under the aerobic condition. Therefore, the distribution of hypoxic cells in the tumor is thought to be critical against a cure rate of radiation therapy. The killing effect of ionizing radiation on cultured mammalian cells was usually judged by the loss of clonogenic activity. Recent studies proved that two types of cell death, necrosis and apoptosis, were main components of the loss of clonogenic activity. The present study was carried out to examine how the hypoxia influences the ionizing irradiation-induced apoptosis in cultured mammalian cells with the aid of a specially designed gas-exchangeable chamber. Human B cells transformed by Epstein-Barr virus (EB-B cells) were employed and exposed to X-rays with 0~24 Gy under the aerobic and hypoxic conditions. Qualitative and quantitative measurements of apoptotic cells were made by the flowcytometric analysis of propidium iodide-stained cells and showed that apoptotic cell death was less induced in hypoxic cells than in aerobic cells. Observation of ladder-like cleavage of DNA, which is

characteristic of apoptosis, in cells irradiated under both conditions further confirmed this phenomenon. Flowcytometric analysis also showed another aspect of X-irradiated cells that the arrest of cell cycle progression at the G1/S and G2/M borders due to X irradiation was weaker in hypoxic cells than in aerobic cells.

To explain these observations, the accumulation of p53 protein and the subsequent decrease in the activity of cell-cycle relating protein kinase cdk2 (cyclin-dependent kinase), which is regulated by the p53 protein, were measured by Western blot analysis and the immunoprecipitation/kinase assay methods, respectively, in X-irradiated aerobic and hypoxic cells. The results showed that X irradiation remarkably induced the accumulation of p53 protein followed by the decrease in the cdk2 activity in aerobic cells, but induced to a less extent in hypoxic cells. Examination of X-irradiated cells by pulse-field gel electrophoresis showed that DNA double-strand breaks were more induced in aerobic cells than in hypoxic cells, suggesting that the difference in the accu-