Abstract

Transmissive spongiform encephalopathies, also called prion disorders, are fatal neurodegenerative diseases affecting mammals. In patients, the pathological prion protein (PrP^{TSE}) accumulates in CNS and causes death. Prions possess high binding affinity to surfaces. Moreover, they are highly resistant to conventional sterilization procedures which rise the risk of nosocomial transmission from patients in subclinical stage of prion disease through medical tools. In the thesis, we evaluate the efficiency of photodynamic inactivation (PDI) for prion decontamination. The PDI is induced by photoactivation of phthalocyanine (Pc) derivates $AlPcOH(SO_3)_2$, $SiPc(OH)_2(SO_3)_{1-3}$ or $ZnPc(SO_3)_{1-3}$. Pc exposed to light generate reactive oxygen species, mainly singlet oxygen ($O_2(^1\Delta_g)$). Production of $O_2(^1\Delta_g)$ in aqueous solution was confirmed by iodide method, quenching by NaN_3 and oxidative degradation of uric acid. The photoactivation of Pc in infectious brain homogenate led to elimination of PrPres signal (= proteinase K-resistant PrP^{TSE} fragment) below the detection limit of western blot by using nanomolar $AlPcOH(SO_3)_2$ concentration. The complete elimination of PrPres signal was accompanied with total protein concentration decrease by a maximum of 20% in brain homogenate No signs of protein fragmentation or aggregation were observed.

The PDI induced by Pc derivatives eliminated PrPres of prion strains ME7, 22L, mBSE, 139A, mvCJD, RML and mFu with various efficiency. The PDI was most efficient when induced by $AlPcOH(SO_3)_2$, followed by $ZnPc(SO_3)_{1-3}$. $SiPc(OH)_2(SO_3)_{1-3}$ was the least effective for induction of PDI.

Reduction of RML prion infectivity induced by PDI was evaluated on mouse model which is extremely sensitive to prion infection. The mouse bioassay revealed that the PDI in presence of $AlPcOH(SO_3)_2$ reduced the infectivity of RML prions by four orders of magnitude. Efficiency of PDI on RML prions bound to plastic surface was monitored by CAD5 cell line susceptible to prion infection. The PDI decreased infectivity of bound RML prion by three orders of magnitude. To summarize, the PDI successfully reduce prion infectivity in suspension and bound to surface. We believe the method can be a powerful tool for prion decontamination if introduced as a part of conventional sterilization procedure in medical facilities.