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황환원제가 재조합 Erythropoietin의 생산과 당쇄구조에 미치는 효과

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Enhancement of the production of recombinant EPO (Erythropoietin) was studied by using Chinese hamster ovary cell. Butyrate, which is known to alter the differentiation of mammalian cells were used to increase the recombinant protein. However, when butyrate was treated in rapidly growing cells, the death of the cells occurred. Intra-nucleosomal DNA fragmentation showed that the death was due to apoptosis. The induction of apoptotic death of the cells could be partially blocked by treatment with the well-known antioxidant, N-acetycysteine (NAC). Strikingly, the NAC treatment enhanced the production of recombinant EPO two-fold, compared with that of the culture without NAC supplementation. These results show that butyrate supplemented with NAC not only inhibited apoptosis, but also exerted a synergistic effect on the production of recombinant EPO. Considering its role as antioxidant, NAC was thought to scavenge Reactive oxygen species (ROS) that might act as signal to apoptosis. However, mechanism of production of enhancing effect is remained to be investigated.

In order to investigate the mechanism for enhancement of production by NAC, effect of thiol reducing agents that have similar effect to NAC, was studied. Mild reducing agents, Mercaptoethansulfonic acid (MESNA), Thiolactic acid (TLA), Thioglycolate (TG) were shown to block apoptosis and also increase the production of EPO. In order to find out whether the effect was general on CHO cells or not, other transformant cells (EC-2 2C5, EC-2 1H9) that produce different amount of EPO were tested. The production enhancing effect was approximately similar in all the cells. The secretion kinetic study using pulse-chase method revealed that all four thiol reducing agents significantly increased the secretion rate of EPO. It was deduced that the thiol reducing agents increased the secretion rate by altering environmental condition

of endoplamic reticulum from the oxidative state to the slightly reduced state and facilitate the folding of the proteins. The morphology of the cells was observed to be altered to irregular shape in presence of thiol reducing agent.

EPO molecule has a complex oligosaccharide structure that plays an important role in biological activity in vitro. To figure out whether thiol reducing agent influence on the glycosylation or not, structural analysis of oligosaccharide was carried out. Cells were cultured in the medium supplemented with 20mM of NAC and then the culture supernatant was withdrawn for purification of EPO. EPO was purified through the procedures employing heparin-sepharose affinity column, anion-exchange (MonoQ) column and gel permeation chromatography (Sephadex G-75) column. The purified EPO was treated with N-glycosidase F, for the isolation of oligosaccharide and the isolated oligosaccharides were labeled with fluorescent dye, 2-aminobenzamide. Labeled oligosaccharide was analyzed with anion-exchange chromatography (MonoQ) and normal-phase chromatography (amide column, Glycosep-N) for the assignment of GU (glucose unit) value. Through the assignment of molecular mass using MALDI-TOF mass spectrometry, final structures of the oligosaccharide were analyzed. As the result, the relative amount of sialylated oligosaccharide in NAC-treated EPO was lower than in EPO obtained from the sample without NAC treatment. This implies that the sialyltransferase activity may not fully support the increased secretion rate of EPO in NAC-treated cells.