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Effects of Mutagenesis for Glycosylation Sites of Recombinant Human EPO During Production from Cultured CHO Cell

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Human eryhropoietin (EPO) is acidic glycoprotein hormone that plays key role in hematopoiesis by facilitating differentiation of erythrocyte and formation of hemoglobin (Hb) and is used for the treatment of anemia. Human EPO is consist of 166 amino acids which is modified by three N-glycosylations (24, 38, 83) and single O-glycosylation (126). N-glycosylation is reported to be related to the cellular secretion and activity of EPO. In this study, we examined effects of mutagenesis in glycosylation site of recombinat hEPO for the cellular secretion during production from cultured CHO cell. We produced rhEpo which was cloned by PCR from human liver cDNA (TaKaRa) in cultured CHO cell. Using supernatant of the culture, ELISA assay and western analysis were performed. To estimate biological activity, 20IU of rhuEpo was subcutaneously injected into four ICR mice. After 8 days, HCT level was increased average 13 per cent, RBC was increased ca. $2\times10^6/\mu\ell$. In disease model Rat (anemia c-kit, WSRC-WS/WS), HCT was increased ca. 12%, RBC was increased ca. $1.6\times10^6/\mu\ell$. These results suggests that rhEpo we produced has biological activity. To remove glycosylation site by substituting 24, 38, 83, and 126th asparagine (or serine) with glutamic acid, overlapping -extension site-directed mutagenesis was performed. To add novel glycosylation sites, 69, 105th leucine was mutated to asparagine. Mutant EPO construct was transfected into CHO cell. Supernatant of the cell culture was analyzed using ELISA assay with monoclonal anti-EPO antibody (Medac, Germany). Since, several reports for mutagenesis of glycosylation sites showed case-by-case results, we examined both transient expression and stable expression. Addition of novel glycosylation sites resulted no secretion while deletion mutants had little effect except some double deletion mutants (24/83 and 38/83) and triple mutant. We suggest that not single but combination of glycosyl group affect secretion of EPO.

Key words) ***hEPO, glycosylation site, site-directed mutagenesis, CHO cell culture, ELISA***