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Enhancement of Foreign Protein Productivity in Recombinant CHO Cells by Inhibition of Cellular Apoptosis under Various Stress Conditions

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Sodium butyrate (NaBu) can enhance the expression of genes from some of the mammalian promoters including CMV and SV40 while it can also inhibit cell growth and induce cellular apoptosis. Thus, the beneficial effect of using a higher concentration of NaBu on a foreign protein expression is compromised by its cytotoxic effect on cell growth. To overcome this cytotoxic effect of NaBu, a survival protein, human Bcl-2, was overexpressed in the recombinant CHO cells (SH2-0.32) producing a humanized antibody directed against the S surface antigen of hepatitis B virus. When batch cultures of both control cells transfected with *bcl-2* deficient plasmid (SH2-0.32-Δbcl-2) and cells transfected with *bcl-2* expression plasmid (14C6-bcl-2) were performed in the absence of NaBu, both cells showed similar profiles of cell viability and antibody production. Compared with SH2-0.32-Δbcl-2 culture, under the condition of NaBu addition at the exponential growth phase, overexpression of *bcl-2* gene considerably suppressed the NaBu-induced apoptosis of 14C6-bcl-2 by inhibiting caspase-3 activity and extending the culture longevity by more than 2 days. As a result, the final antibody concentration of 14C6-bcl-2 culture was 2 times higher than that of SH2-0.32-Δbcl-2 culture in the presence of NaBu and 3 times higher than that of SH2-0.32-Δbcl-2 and 14C6-bcl-2 cultures in the absence of NaBu.

Overexpression of human Bcl-2 protein in recombinant Chinese hamster ovary (rCHO) cells producing humanized antibody (SH2-0.32) considerably suppressed sodium butyrate (NaBu)-induced apoptosis during batch culture using a commercially available serum-free medium, extending the culture longevity. Due to the enhanced transcription efficiency endowed by NaBu and the extended culture longevity provided by anti-apoptotic effect of Bcl-2 overexpression, the final antibody concentration of 14C6-bcl-2 culture was 2 times higher than that

of SH2-0.32-Δbcl-2 culture (cells transfected with *bcl-2*-deficient plasmid) in the presence of NaBu. To determine the effect of NaBu/Bcl-2 overexpression on the molecular integrity of protein products, antibodies purified from 14C6-bcl-2 and SH2-0.32-Δbcl-2 cultures in the presence of NaBu were characterized using various molecular assay systems. For comparison, antibody purified from parental rCHO cell culture (SH2-0.32) in the absence of NaBu was also characterized. No significant changes in molecular weight of antibodies could be observed at the level of SDS-PAGE. From GlycoSep-N column analysis, it was found that the core oligosaccharide structure (GlcNAc2Man3GlcNAc2) was not affected by NaBu/Bcl-2 overexpression while the microheterogeneity of N-linked oligosaccharide structure was slightly affected. Compared with the antibody produced in the absence of NaBu, the proportion of neutral oligosaccharides was increased by 10% (14C6-bcl-2) to 16% (SH2-0.32-Δbcl-2) in the presence of NaBu, which was accompanied by the reduced proportion of acidic oligosaccharides, especially of monosialylated and disialylated forms. The changes in microheterogeneous oligoformal structures of antibody in turn affected the mobility of antibody isoforms in isoelectric focusing (IEF), resulting in the occurrence of some more basic antibody isoforms produced in the presence of NaBu. However, the antigen-antibody binding properties were not changed by alteration in glycosylation pattern. The competitive enzyme-linked immunosorbent assay (ELISA) showed that the antibody produced by NaBu/Bcl-2 overexpression maintained its antigen-antibody binding properties with binding affinity of about $2.5 \times 10^{9}\ M^{-1}$. Taken together, no significant effects of NaBu/Bcl-2 overexpression on the molecular integrity of antibodies produced using serum-free medium could be observed by the molecular assay systems.

Apart from the overexpresson of Bcl-2 survival protein to overcome cytotoxic effect of NaBu, the expression vector of antisense RNA of caspase-3 was also constructed and transfected into SH2-0.32 cells. Using this antisense RNA strategy, rCHO cells (B3) producing a low-level of caspase-3 proenzyme were established. When batch cultures of both B3 cells and control cells transfected with antisense RNA-deficient plasmid were performed in the absence of NaBu, both cells showed similar profiles of cell growth and antibody production.

Compared with control cell culture, under the condition of 5 mM NaBu addition at the exponential growth phase, expression of antisense RNA of caspase-3 significantly suppressed the NaBu-induced apoptosis of B3 cells and extended culture longevity by more than 2 days. However, compared with control cell culture, the final antibody concentration of B3 cell culture was not increased in the presence of NaBu, which may be due to the loss of cellular metabolic capability resulted from the depolarization of mitochondrial membrane. Taken together, this study suggests that, although expression of antisense RNA of caspase-3 does not improve antibody productivity of rCHO cells, it can suppress NaBu-induced apoptotic cell death of rCHO cells and thereby may reduce problems associated with cellular disintegration.