Glycosylation variant analysis of recombinant Human Tissue plasminogen activator produced in urea cycle enzyme expressing CHO cell line

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Abstract

tPA were produced in OTC-tPA cells and by introducing tissue plasminogen activator (tPA) into OTC cells. OTC cells, originally drived from CHO cells which express the first two enzymes of the urea cycle, carbamoyl phosphate synthetase Ⅰ (CPS Ⅰ) and ornithine transcarbamoylase (OTC), had been known for enhancing erythropoietin production (1,2). tPA glycosylation variants produced in OTC-tPA cells, were separated by Lysine Sepharose 4B chromatography, analyzed, and determined for their biological activity. Two major peak obtained by Lysine Sepharose 4B chromatography, type Ⅰ Ⅱ (complex type) and type Ⅱ variants. The ratio of type Ⅰ variant and type Ⅱ variant was analyzed using ELISA, SDS-PAGE and Western blot. The biological activity of tPA produced in OTC-tPA cells was determined by unstimulated indirect amidolytic assay. As a result of glycosylation variant analysis, ratio of type Ⅰ variant and type Ⅱ variant were 23 % and 77 %, respectively. The biological activities of type Ⅰ Ⅱ variant and type Ⅱ variant were two times more than that of 3rd international tPA standard (98 / 713). The biological activity of type Ⅱ variant was 12.6 % more than that of type Ⅰ Ⅱ variants. Therefore, type Ⅱ variant contained more superior biological activity. These results demonstrate that the introduction of urea cycle enzymes into CHO cells could enhance not only the biological activity of tPA but also more production of type Ⅱ variant which is very valuable for its production in biopharmaceutical industry.

Reference

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