

Modification of the goat mammary gland glycosylation pathway by overexpression of GnT-IVa

María José Leiva Carrasco

Biotechnology and Biopharmaceuticals Laboratory, Pathophysiology Department, School of Biological Sciences, Universidad de Concepción, Chile

Abstract

Statement of the Problem: Recombinant glycoprotein expression has been carried out in different expression systems based on the genetic modification. Specifically, recombinant glycoproteins whose biological activity depends on post-translational modifications are produced in cell tissue culture, which increases the manufacturing cost. The production of biotherapeutics in the mammary glands of genetically modified mammals results in an alternative method to overcome the drawback of cell culture expression system. However, the N-glycosylation pattern of complex glycoproteins produced in the mammary epithelia has showed diminished antennae formation and lower sialic acid contents compare to native protein. An alternative to obtain high- quality biopharmaceuticals in milk could be the modification of the N-glycosylation pattern by overexpression of exogenous glycosyltransferases. The purpose of this study is to modify in vivo the glycosylation pattern of recombinant protein expressed in goat mammary gland. Methodology & Theoretical Orientation: Human erythropoietin fused to human IgG Fc (EPO-Fc) was co-expressed with N-acetyl-glucosaminyltransferase-IVa (GnT-IVa) by adenoviral transduction in goat mammary gland. Findings: The modification in vivo of the enzymatic glycosylation machinery in the mammary gland generated an increment in the antennae number. A higher population of tri-antennary structures for the EPO-Fc/GnT-IV variant was obtained by N-glycans mass spectrometry analysis, compared to bi-antennary structures N-linked to EPO-Fc expressed in the same cells. Conclusion & Significance: These results demonstrate, for the first time, that it is possible to modify in vivo the glycosylation pattern of recombinant biopharmaceutical expressed in the goat mammary gland epithelial cells to obtain a glycosylation pattern similar to native glycoproteins.

Keywords: Biochemistry, Glycoprotein

Biochemistry is both life science and a chemical science - it explores the chemistry of living organisms and the molecular basis for the changes occurring in living cells. It uses the methods of chemistry,

"Biochemistry has become the foundation for understanding all biological processes. It has provided explanations for the causes of many diseases in humans, animals and plants."

physics, molecular biology, and immunology to study the structure and behaviour of the complex molecules found in biological material and the ways these molecules interact to form cells, tissues, and whole organisms.

Biochemists are interested, for example, in mechanisms of brain function, cellular multiplication and differentiation, communication within and between cells and organs, and the chemical bases of inheritance and disease. The biochemist seeks to determine how specific molecules such as proteins, nucleic acids, lipids, vitamins, and hormones function in such processes. Particular emphasis is placed on the regulation of chemical reactions in living cells.