PREPARATION AND INTEGRITY EXAMINATION OF FREEZE DRIED KIT OF TRASTUZUMAB-IMMUNOCONJUGATES AND COLD LABELED IMMUNOCONJUGATES BY APPLYING SDS-PAGE

ELECTROPHORESIS

Abstract

Radioimmunoconjugates are promising agents in diagnostic and treatment of different types of

cancers. The aim of this study is formulation of stable freeze dried kits of trastuzumab with three

types of bifunctional chelators for further radiolabeling. The integrity of the antibody in formulated

conjugates was examinated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-

PAGE).

Trastuzumab is a humanized monoclonal antibody used in the therapy of aggressive HER2

positive breast cancer. Conjugation of trastuzumab was made with various chelators: p-SCN-Bn-DTPA

(1:10, 1:20, 1:50), p-SCN-Bn-DOTA (1:20), and 1B4M-DTPA (1:10, 1:20, 1:50). The purified

immunoconjugates were lyophilized by applying two day protocol in order to produce the stable freeze

dried kits. Cold labeling with nonradioactive isotopes LuCl3 and YCl3 was performed to view the

possible modifications of secondary structure after radioactive labeling. SDS-PAGE electrophorezis

under reducing conditions was used to estimate the purity and integrity of the antibody before and

after conjugations, lyophilization and labeling.

To conclude with, the results show that there is no degradation of the examined antibody. The

trastuzumab-conjugates and cold labeled formulations migrated in two bands (~50kDa and ~25kDa),

in the same way as IgG1 antibodies and unmodified trastuzumab.

Key words: bifunctional chelators, conjugations, electrophoresis, trastuzumab