Development of Specific ELISAs for Phase II Drug Metabolizing Glutathione Transferase A Class Isoforms.

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Abstract

Antibodies first successfully reflect organ damage by mediating the activation of a large variety of beneficial and damaging agents are crucial for drug development and for detecting and reducing the occurrence of diseases resulting from environmental and genetic causes. Human glutathione-S-transferases (GSTs) are a superfamily of enzymes that catalyze the conjugation of glutathione (GSH) with electrophilic xenobiotics and reactive byproducts of normal metabolism, providing protection against tissue injury. High-cytoxic conjugations and a resulting shift in balance. In addition, it should be sensitive enough to detect the early increase of GST A in blood samples, for instance, could be an indication of toxicity from exposure to an environmental or toxic xenobiotic. Our goal to develop a panel of highly sensitive GST immunoassays using antibodies specific for not only A Class but also for other GSTs and under different conditions of assay, with emphasis on the anti-GST A3 ELISA.

Introduction

An ideal labelling of organ damage responsive proteins to identify tissue localization. High-cytoxic conjugations and a resulting shift in balance. In addition, it should be sensitive enough to detect the early increase of GST A in blood samples, for instance, could be an indication of toxicity from exposure to an environmental or toxic xenobiotic. The hybridoma supernatants were incubated in wells then washed 3 times with in 2x PBS and blocked with 10% FBS in 2x PBS. The wells were washed once more before the addition of an HRP-conjugated secondary antibody at a concentration of 0.04 mg/ml. Owing to the large number of GST antigens present, a specific signal was observed only for mouse-anti human GST A class antigens. The following table summarizes the antibodies currently in-house at Oxford Biomedical Research. The following table summarizes a typical EIA that was performed to determine the specificity and sensitivity of this antibody. The assay was developed using a purified mouse anti-A3-3 monoclonal antibody with a 1:500 dilution in 5% BSA/1% normal goat serum/0.05% Tween 20 in PBS. The specificity of the monoclonal antibody was determined by using recombinant human GST A3-3 (whole molecule).

Evaluation of GST A Isoform-specific antibodies

To determine the specificity and sensitivity of these antibodies, recombinant human GST A3-3 (Whole molecule) and recombinant human GST A3-4 (Whole molecule) were used as the standard. The specificity was determined by using recombinant human GST A3-3 (Whole molecule) and recombinant human GST A3-4 (Whole molecule) as the standard. The specificity was determined by using recombinant human GST A3-3 (Whole molecule) and recombinant human GST A3-4 (Whole molecule) as the standard.

Results & Findings

A range of monoclonal and polyclonal antibodies have been developed in a collaborative effort with antibody production facilities (that exhibit specificities for human GST A class isoforms. Among these antibodies are those that are specific for only one A Class isoform. The following table summarizes a typical EIA that was performed to determine the specificity and sensitivity of this antibody. The assay was developed using a purified mouse anti-A3-3 monoclonal antibody with a 1:500 dilution in 5% BSA/1% normal goat serum/0.05% Tween 20 in PBS. The specificity of the monoclonal antibody was determined by using recombinant human GST A3-3 (Whole molecule).

Antibodies against human GST A Class isoforms

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References

14. Oxford Biomedical Research, Rochester Hills, MI.