

Determination of Aldehyde Dehydrogenase (ALDH) Isozymes in Human Cancer Samples - Comparison of Kinetic and Immunochemical Assays

Abstract

A fluorimetric assay of aldehyde dehydrogenase isozymes, based on naphthaldehyde oxidation, is compared with Western Blotting analysis on several clinical samples obtained from surgery. The comparison reveals qualitatively good correlation of ALDH1A1 isozyme detection with two methods and somewhat worse on ALDH3A1 assay. It has been confirmed that two cytosolic ALDH isozymes, ALDH1A1 and ALDH3A1 (formerly known as ALDH-1 and ALDH-3) are those primarily responsible for drug inactivation. The activity levels of the foregoing isozymes also reveal some predictive potential for cancer metastasis level. Evaluation of ALDH activity levels in clinical material by traditional spectrophotometric approach, i.e., using either acetaldehyde or propionic aldehyde as a substrate, and measuring NADH production rate, is not isozyme-specific and usually requires prior separation of the enzymes from the clinical sample.

Keywords: ALDH isozymes • aldehyde concentrations • isozyme • macromolecule

Introduction

An omnipresent, polymorphic catalyst organic compound dehydrogenase (ALDH, E.C.1.2.1.3) plays a very important role within the method of inactivation of the many xenobiotic, together with anti-cancer medication of the oxazaphosphorine series, in living cells. It's been confirmed that 2 cytosolic ALDH isozymes, ALDH1A1 and ALDH3A1 (formerly referred to as ALDH-1 and ALDH-3) are unit those primarily chargeable for drug inactivation [1]. The activity levels of the preceding isozymes conjointly reveal some prognosticative potential for cancer metastasis level. Analysis of ALDH activity levels in clinical material by ancient spectrophotometric approach, i.e., exploitation either aldehyde or propionic organic compound as a substrate, and measure NADH production rate, isn't isozyme-specific and frequently needs previous separation of the enzymes from the clinical sample. Far better specificity is obtained with chemistry strategies, primarily ELISA, evaluating macromolecule content, rather than its activity. It's conjointly been postulated that a completely unique fluorimetric assay, supported reaction of fluorescent and fluorogenic naphthaldehydes exhibits far better property for the cytosolic ALDH forms, and so is also applied directly for crude tissue homogenates [2].

Naphthaldehyde activities, according during this paper, are unit appreciate a lot of commonly used aldehyde activities, if aldehyde concentrations don't exceed two hundred two hundred. Variations between growth and tissue activities have conjointly been according for alternative varieties of cancer and should have each diagnostic and therapeutic significance. There is a decent qualitative correlation of Western Blot analysis and also the results of activity, particularly for ALDH1A1 isozyme in colon tissue. Somewhat higher variability of the activity measurements will be explained by high susceptibility of this isozyme to inactivation by medication, alcohol, and alternative factors. At intervals the colon samples, there's conjointly quite smart correlation of ALDH3A1 determinations

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by 2 strategies, however this correlation is way worse for alternative varieties of tissue. This could be thanks to the polymorphism of ALDH-3 kind (dimeric) inducible isozymes [3].

Description

We have conferred proof confirming the specificity of the fluorimetric ALDH assay, utilizing 7-methoxy- 1-naphthaldehyde as a substrate, toward the cytosolic ALDH1A1 isozyme within the clinical samples, by showing correlation between fluorimetric and Western Blot analyses. Somewhat worse, however still satisfactory specificity is found for the fluorimetric ALDH3A1 assay. The fluorimetric methodology will be accustomed fast screening of ALDH activity in clinical material Naphthaldehyde substrates and also the corresponding carboxylates were synthesized as antecedently delineated and every one reagent were from letter or Aldrich. Water was filtered through a Milli-Q system. Samples of neoplasm tissue further as management benign tissues obtained from the neoplasm patients were homogenized

and solicited in an exceedingly RIPA buffer supplemented with PI cocktail tablets (Roche Diagnostics GmbH, Mannheim, Germany), Na orthovanadate and PMSF [4].

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Conflict of Interests

None

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