Application of a Galactosidase Immunosorbent Test to Carcinoembryonic Antigen in Plasma

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SUMMARY

The galactosidase immunosorbent test for carcinoembryonic antigen is simple to perform, uses stable reagents, does not require radioactive reagents, and is adaptable to large numbers of samples. Concentration of carcinoembryonic antigen in sera or plasma was determined by the galactosidase immunosorbent test and by the Egan-Todd double antibody assay (93% agreement), indirect Z-gel (83% agreement), and direct Z-gel assay ($\rho = 0.97$). The galactosidase immunosorbent test has potential as a clinically useful nonisotopic assay for carcinoembryonic antigen.

INTRODUCTION

Measurement of CEA concentration in body fluids is a valuable clinical tool (9, 10, 14). CEA radioimmunoassays developed by Thompson et al. (15), by LoGerfo et al. (8), and by Egan et al. (3) correlate well with each other and have yielded important insights into the structure of CEA and the clinical significance of CEA in adenocarcinoma of the large bowel and other neoplastic and nonneoplastic diseases (10, 14).

We have developed a galactosidase immunosorbent test for CEA (4, 5, 16) which is made possible by use of antibody conjugates of $\beta$-galactosidase. The galactosidase immunosorbent test for CEA has several desirable characteristics: the use of unstable radioactive reagents is bypassed; the antibody-enzyme conjugate is stable in solution for at least 1 year; and the immunoadsorbent eliminates the need to centrifuge a gel or immune precipitate and permits simple aspiration of fluids from the solid phase. We present here a comparison of the galactosidase immunosorbent test with some widely used radioimmunoassays for CEA.

MATERIALS AND METHODS

Quantitation of CEA by Galactosidase Immunosorbent Assay. Quantitation of CEA by the galactosidase immunosorbent test was performed as described previously (4), except that rabbit anti-CEA Serum 351 was used as the solid-phase immunosorbent. Sample volume for assay varied from 1 to 500 $\mu$L. Plasma samples were obtained from EDTA-treated whole blood.

Quantitation of Serum CEA by Radioimmunoassay. CEA levels in sera were determined by Todd-Egan double antibody radioimmunoassay (3) as modified by Laurence et al. (7), using goat anti-CEA serum (Peanuts 12). CEA levels in plasma were determined either by indirect or direct Hansen Z-gel radioimmunoassay (6), as indicated below.

RESULTS

The galactosidase immunosorbent test was compared with widely used radioimmunoassays for CEA (Table 1). A comparative study of CEA in normal serum was undertaken with the galactosidase test and the Todd-Egan double antibody radioimmunoassay. Table 1 gives the mean CEA concentrations for 26 normal sera, each determined in duplicate by both assays. The cutoff points for normal serum CEA concentration, 3 standard deviations above the mean, were 9.1 and 8.4 ng/ml for the galactosidase and Todd-Egan assay, respectively. A comparative study of CEA in perchloric acid extracts of normal plasma was undertaken with the galactosidase immunosorbent test and the indirect Z-gel assay. The cutoff points for normal plasma CEA concentration, 3 standard deviations above the mean, were 10.8 and 3.4 ng/ml for the galactosidase and indirect Z-gel, respectively.

CEA was measured in plasma and sera of patients with a variety of neoplastic and nonneoplastic diseases by means of the galactosidase assay and radioimmunoassay. As shown in Table 2, there were no false positives by galactosidase assay relative to the indirect Z-gel assay of 52 perchloric acid plasma extracts. As shown in Table 3, there was a 1.8% incidence of false positives in the galactosidase assay relative to the Todd-Egan method. Furthermore, there was 92.8% concordance between the galactosidase and indirect Z-gel assays and 85.7% concordance between the galactosidase and Todd-Egan methods (Tables 2 and 3).

Plasma samples with elevated CEA concentrations were assayed by the direct Z-gel method (6) and by the galactosidase immunosorbent test (Table 4). There were no false negatives for CEA by the galactosidase immunosorbent test. The mean ratio of CEA concentration determined by the galactosidase immunosorbent test to that determined by Z-gel was 0.92; ratios ranged from 0.33 to 1.80. Linear regression analysis of the data in Table 4 was performed. The slope of the regression line was 1.09, the intercept was $-33$ ng/ml, and the linear correlation coefficient was 0.997.
Table 1

<table>
<thead>
<tr>
<th>Material</th>
<th>No. of samples</th>
<th>Test</th>
<th>CEA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal sera</td>
<td>26</td>
<td>Galactosidase</td>
<td>3.1 ± 2.0 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Todd</td>
<td>3.3 ± 1.7</td>
</tr>
<tr>
<td>Normal plasma a</td>
<td>28</td>
<td>Galactosidase</td>
<td>6.8 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z-Gel</td>
<td>1.6 ± 0.6</td>
</tr>
</tbody>
</table>

a Mean ± S.D.

Normal plasma was extracted with 0.6 M perchloric acid and dialyzed against water and 0.01 M ammonium acetate, according to the method of Hansen et al. (6).

Table 2

Comparison of the galactosidase immunosorbent test and indirect Z-gel assay for CEA

<table>
<thead>
<tr>
<th>Enzyme:indirect Z-gel</th>
<th>Comment</th>
<th>No. of individuals</th>
<th>% of total individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>+:+</td>
<td>Concordant high titer</td>
<td>12</td>
<td>23.1</td>
</tr>
<tr>
<td>-:+</td>
<td>False-negative galactosidase immunosorbent test</td>
<td>9</td>
<td>17.3</td>
</tr>
<tr>
<td>+:-</td>
<td>False-positive galactosidase immunosorbent test</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-:-</td>
<td>Concordant low titer</td>
<td>31</td>
<td>59.6</td>
</tr>
</tbody>
</table>

+ : CEA concentration either >10.8 or >3.4 ng/ml by galactosidase immunosorbent test or indirect Hansen Z-gel assay, respectively. Plasma samples were extracted with 0.6 M perchloric acid and dialyzed according to the method of Hansen et al. (6) prior to galactosidase and Z-gel assays. -, CEA concentration either <10.8 or <3.4 ng/ml by galactosidase immunosorbent test or indirect Hansen Z-gel assay, respectively.

Table 3

Comparison of the galactosidase immunosorbent test and Todd assay for CEA in sera

<table>
<thead>
<tr>
<th>Enzyme:Todd</th>
<th>Comment</th>
<th>No. of individuals</th>
<th>% of total individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>+:+</td>
<td>Concordant high titer</td>
<td>2</td>
<td>3.6</td>
</tr>
<tr>
<td>-:+</td>
<td>False-negative galactosidase immunosorbent test</td>
<td>3</td>
<td>5.4</td>
</tr>
<tr>
<td>+:-</td>
<td>False-positive galactosidase immunosorbent test</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>-:-</td>
<td>Concordant low titer</td>
<td>50</td>
<td>89.2</td>
</tr>
</tbody>
</table>

+ : CEA concentration either >9.1 or >8.4 ng/ml by galactosidase immunosorbent test or Todd assay, respectively. -, CEA concentration either <9.1 or <8.4 ng/ml by galactosidase immunosorbent test or Todd assay, respectively.

DISCUSSION

The galactosidase immunosorbent test for CEA (4) has several desirable features: stable antibody-enzyme conjugates are used rather than unstable radioactive reagents; use of an immunoadsorbent eliminates the need to centrifuge a gel or immune precipitate and permits direct aspiration of fluids from the solid phase; assay of the amount of enzyme-conjugate adsorbed to insolubilized CEA-anti-CEA complexes may be either colorimetric or fluorimetric (11, 12). Furthermore, in competition radioimmunoassays for CEA, increased amounts of CEA result in decreased amounts of radioactivity counted. In contrast, in the galactosidase immunosorbent test increased amounts of CEA result in increased amounts of detected enzymatic activity (4). While competition immunoassays for CEA achieve only partial standard curve linearization using logarithmic or logit transformations (2), the galactosidase immunosorbent test for CEA achieves complete standard curve linearization through use of a simple double reciprocal plot of enzymatic activity versus CEA added (4). Linearity of the double reciprocal plot simplifies regression line fitting, permits accurate

(p < 0.01). However, the variance of CEA concentration determined by the galactosidase immunosorbent test was proportional to CEA concentration determined by Z-gel, i.e., the variance was heteroscedastic. Therefore, a nonparametric method was used to analyze the data. The values in Table 4 were ranked by CEA concentration, and linear regression analysis on the ranked data was performed. The slope of the regression line was 0.97, the intercept was 1.23, and the Spearman rank correlation coefficient was 0.97 (p < 0.01). The ranked data and the calculated regression line are shown in Chart 1.
interpolation between data points, and readily identifies an erroneous data point.

CEA concentrations determined by galactosidase immunosorbent test in sera of 26 putatively normal individuals suggest that samples with CEA concentrations greater than 9.1 ng/ml should be considered abnormal. The galactosidase immunosorbent test showed 83 and 93% concordance with the indirect Z-gel (Table 2) and Todd-Egan (Table 3) assays, respectively. This is comparable to the degree of agreement between the Thompson-Gold and the Hansen CEA radioimmunoassays (83 to 87%) (13) and between the Hansen and the Todd CEA radioimmunoassays (72%) (7). The galactosidase immunosorbent test and the direct Hansen Z-gel CEA assay showed good overall agreement on 71 plasmas with grossly elevated CEA levels (Table 4; Chart 1). However, although the Spearman rank correlation coefficient of 0.97 is high ($p < 0.01$), the ratios of CEA concentration determined by galactosidase immunosorbent test to that determined by Z-gel ranged from 0.33 to 1.80 (Table 4). This range of ratios indicates that some plasma samples were discordant, in that they were either underestimted or overestimated by the galactosidase immunosorbent test relative to Z-gel. Some of the discordance shown in Tables 2 to 4 may reflect immunoochemical differences between the galactosidase immunosorbent test and the radioimmunoassays for CEA.

The antisera used for the galactosidase assay react with normal cross-reacting antigen and other molecules in plasma, in addition to authentic CEA (4). Some of the differences in mean CEA values shown in Table 1 are probably due to the cross-reacting molecules. The galactosidase test was performed with whole normal serum and with normal plasma that had been extracted with 0.6 M perchloric acid. It is not clear why extraction with perchloric acid caused an increase in apparent CEA-like activity in normal plasma (Table 1). However, we have shown that much of the CEA-like activity in unextracted plasma is not due to authentic CEA (4). In spite of these cross-reactions, however, there was reasonable correlation between the galactosidase and radioimmunoassays. In the present form, the precision and sensitivity of the galactosidase immunosorbent test are comparable to those of the Hansen Z-gel and Egan-Todd radioimmunoassays for CEA (Table 5). However, future studies must be performed with appropriately absorbed antisera in order to increase immunospecificity for CEA (4).

There are still some unsolved problems with the galactosidase assay for CEA. The galactosidase immunosorbent test for CEA, as described here, requires 3 overnight incubations. However, we have found that use of a fluorogenic enzyme substrate reduces the time of assay for enzyme-antibody conjugate by over 400-fold. Use of fluorogenic substrate makes possible a significant reduction in time required for CEA assay by the galactosidase immunosorbent method. Comparisons between the galactosidase and Todd assays for CEA (Tables 1 and 3) suggest that the galactosidase assay may be satisfactorily performed directly on whole-body fluids. We have not yet carried out a full comparison between the indirect Z-gel and galactosidase assays. However, preliminary data that we have obtained indicate that elimination of cross-reactions by absorption of antisera with insolubilized normal cross-reacting antigen significantly increases immunospecificity. Thus, it is hoped that use of absorbed anti-CEA from 2 different species in a sandwich (4) may eliminate need for extraction of samples with perchloric acid. Increased immunospecificity and shortened time are necessary in order to make practicable the clinical application of the galactosidase method for CEA quantitation.

ACKNOWLEDGMENTS

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REFERENCES

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