proteinase properties, for example, alpha-1-antitrypsin. On the possibility that the additional isoenzyme, or one or more of these proteins, has the effect just described, we are carrying out further investigations to try to elucidate this.

References


Sharon Gordon*
Graham Cooper
William Murray*

Depts. of Biochem.* and Surgery*
Western Infirmary
Glasgow, G11 6NT U.K.

Rosalind Campbell
Simon Oakes

Dept. of Pathol. Biochem.
Glasgow Royal Infirmary
Glasgow, G4 OSF U.K.

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Radioimmunoassay Used to Monitor Therapy with Testosterone

To the Editor:

Radioimmunoassay (RIA) for testosterone is used in many laboratories to monitor therapy with testosterone. Traditionally, a preliminary extraction into organic solvent is required, to isolate the steroid and separate it from its binding proteins. Kits not requiring such extraction are now being marketed. We compared two such assays, BIO-RIA's "125I-Testosterone" and Mallinkrodt's "RIA-mat Testosterone" with a method that requires extraction, Amersham's "Testosterone/Dihydrotestosterone RIA" kit. The assays were all done according to the manufacturers' instructions. In investigating the patients being treated with testosterone, we also performed the BIO-RIA assay on plasma extracted with dichloromethane in a manner identical to that used for the Amersham method.

Although we found good intercorrelation of the assay results when measuring endogenous testosterone (correlation coefficients of 0.90 to 0.94), results disagreed if the patients were being treated with testosterone analogs, methyl testosterone or testosterone oenanthalate (Table 1).

<table>
<thead>
<tr>
<th>Testosterone, nmol/L</th>
<th>BIO-RIA</th>
<th>Amersham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl testosterone</td>
<td>&gt;69</td>
<td>&gt;69</td>
</tr>
<tr>
<td>&gt;69</td>
<td>48.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Testosterone oenanthate</td>
<td>69</td>
<td>5.4</td>
</tr>
<tr>
<td>&gt;69</td>
<td>21</td>
<td>32.3</td>
</tr>
<tr>
<td>&gt;69</td>
<td>1.8</td>
<td>29.3</td>
</tr>
<tr>
<td>18.3</td>
<td>3.2</td>
<td>19.1</td>
</tr>
</tbody>
</table>

Plasma from patients receiving methyl testosterone gave high values with BIO-RIA reagents. The fact that the values for extracted plasma were of the same order as the direct values shows that the drug was extracted. Hence, the low values found with the Amersham reagent must be related to a lower affinity of the Amersham antibody for methyl testosterone.

The case of testosterone oenanthate is more complicated. Very high values were obtained with the direct BIO-RIA assay, but low values were obtained with the same reagent for extracted plasma. Evidently the plasma contains non-extractable metabolites with an affinity for the BIO-RIA antibody.

The Amersham kit values for extracted plasma were higher than the BIO-RIA values for extracted plasma. This shows a greater cross-reactivity of the Amersham antibody for extracted metabolites. However, the Amersham results were lower than the direct BIO-RIA values for plasma. Thus there are both extraction and antibody cross-reactivity problems with testosterone oenanthate. We do not know which of these metabolites, extractable or non-extractable (or both), are biologically active, and to what extent each cross-reacts in the assay.

Further studies with testosterone oenanthate, testosterone beta-glucuronide, and 17a-methyl testosterone (obtained from Sigma Chemical Co.) showed that the presence of the large oenanthate and glucuronide groups on the molecule blocked the reaction with the antibodies from the three sources we examined. The 17a-methyl group hindered the reactivity of the antibodies to different extents.

The curves obtained with BIO-RIA reagents for testosterone and methyl testosterone were superimposable. The binding curve for methyl testosterone with Amersham reagents was almost horizontal, showing low antibody affinity and confirming the patients' data. The binding curve for methyl testosterone with Mallinkrodt reagents paralleled that of testosterone but showed lower affinity for the drug. This means that measured results for testosterone in the plasma of patients treated with methyl testosterone would differ among the three different sets of reagents used.

We conclude that the values for testosterone obtained by RIA for patients on replacement therapy may not have the same clinical significance as the values for endogenous testosterone.

Sujiva Ratnaike
David Campbell
Max Goodwin

Dept. of Biochem.
The Royal Melbourne Hosp.
Grattan St.
Parkville, Victoria, 3050
Australia

Lactate Dehydrogenase and Its Isoenzyme, LDH-1, in Serum Are Markers of Testicular Germ Cell Tumors

To the Editor:

The observations of Vladutiu (1) do not allow any conclusions as to the histological pattern of testicular germ cell tumors from the finding of increased LDH (1.1.1.27) and (or) LDH-1 (only) in serum. This agrees with our experience (2). We found no statistically significant difference between LDH activity in the serum of 11 patients with lesions from seminomas, five patients with embryonal carcinoma, or 21 with other nonseminomatous testicular germ cell tumors. Of 11 patients with seminomas, five had increased LDH but only one had increased LDH-1 (the only increased isoenzyme, 72% of the 920 U/L total) in the serum. Of 26 patients with nonseminomatous testicular germ cell tumors, 10 had increased LDH and, of these, only three had increased LDH-1 only. One of these with seminoma + embryonal carcinoma + yolk sac tumor had 63% LDH-1 of the total 610 U of LDH per liter, and one with seminoma + embryonal carcinoma + immature teratoma had 59% of the 680 U/L total. The third patient, with lesions from only yolk sac tumor of the testis, had LDH-1 that was 87% of the 570 U/L total LDH in serum collected from a peripheral arm
Response to a Criticism of the Du Pont CK-MB Test

To the Editor:
The recommendations for creatine kinase (EC 2.7.3.2) isoenzyme MB (CK-MB) testing made by Drs. Stein and Bohner (Clin Chem 30: 238–242, 1984) are consistent with those Du Pont has included in the CK-MB product labeling (1, 2) and educational literature (3, 4) since the method was introduced. However, Du Pont based these recommendations not on matrix effects but on more fundamental principles. For instance, adherence to a sequential sampling protocol is important for any CK-MB method because of the characteristic time sequence followed by CK-MB after a myocardial infarction. By checking for this time sequence, one can make the CK-MB test more specific for myocardial infarction; cases in which CK-MB is present owing to other disease states can usually be identified because of the absence of this time curve. It is an additional benefit that matrix effects, such as interference from atypical CK isoenzymes, can usually be sorted out by sequential analysis.

The recommendation to confine CK-MB testing to a population of patients suspected of myocardial infarction and with CK activities above normal has its basis in predictive value theory and applies to any laboratory test. Following both of these guidelines has the effect of increasing the prevalence of disease in the test population and thus reduces the chance of false positives.

The recommendation not to dilute samples has been stated in the CK-MB product labeling since the method was introduced. However, the assay range (0–350 U/L) is wide enough to allow for direct analysis in most cases, and if activity is greater than 350 U/L it is usually acceptable to report the result as “greater than 350 U/L.”

The possibility of interferences from atypical isoenzymes was documented during field evaluation of the CK-MB method (2), and statements to this effect as well as guidelines for identifying this type of interference have always been included in the product labeling.

Drs. Stein and Bohner correctly point out that ion-exchange chromatography can be sensitive to the sample matrix, and the effects they have demonstrated are consistent with known principles of ion-exchange chromatography. However, in this study the matrix effects are likely to be enhanced with respect to human serum. Some reasons for this follow.

The process of purification of CK isoenzymes can affect the tertiary structure and the charge density of these proteins and alter their behavior on an ion-exchange column. In our experience with the Du Pont CK-MB method, we have observed that purified CK isoenzymes do not always mimic the native species. Therefore conclusions drawn from studies on specimens with purified CK isoenzymes should not be applied to performance of the CK-MB method in the clinical setting.

In the experiments designed by Stein and Bohner to show the effect of protein concentration on the analytical recovery of CK-MB (their Figures 5.1 and 5.2) the CK-MB method was challenged with samples supplemented with a purified CK-MB isoenzyme fraction to give 550 U/L, an activity well above the assay range (0–350 U/L). Performance of the CK-MB test with this model is unlikely to represent performance at the decision level of 10 U/L, nor are samples of this nature likely to be encountered in normal clinical application of the CK-MB test.

In two experiments the analytical recovery of CK-MB was estimated to be 68 to 78%. In one experiment with patients’ samples this estimate (78%) was based on comparison of the Du Pont CK-MB results with those of the immunoinhibition method (25°C), after temperature correction. In the second experiment with samples supplemented with purified CK-MB, the estimate (67%) was based on comparison with the immunoinhibition method at 37°C. However, in neither case is evidence presented to document that the immunological method is giving 100% recovery, and therefore an equally valid interpretation of the data is that the immunological method overestimates CK-MB. Also, the reagent formulation of the assay for CK activity, especially as regards the activator, will profoundly affect the enzyme activity, as is well documented. Given these different formulations of the two CK assays, it is highly unlikely that equivalent amounts of enzyme would give equal activity. Therefore, even if recovery were equal between methods, results should not be expected to be directly comparable, even after temperature correction; the bias between assays can be due entirely to differences in reagent formulation. It is important to point out that an accurate estimate of recovery is not a critical factor in assessing the diagnostic accuracy of the Du Pont CK-MB method. The diagnostic criteria that Du Pont recommends are based on empirical determinations in populations of normal and hospitalized patients, using a recovery estimate of 52%. Therefore, if any bias exists, it is built into the diagnostic criteria, and determination of recovery becomes an academic issue, not a practical one.

References

F. E. von Eyben
Dept. of Intern. Med.
The County Hosp. in Nakskov
DK-4900 Nakskov
Denmark

G. Skude
Dept. of Clin. Chem.
Kalmar Country Hospital
S-391 85 Kalmar
Sweden