isofoms in the repurfused patients was from two- to fourfold greater than in the group of conservatively treated AMI patients. Therefore, in agreement with the conjecture of Wu, the rate of increase of the different CK isofoms in serum is useful as an early indicator of successful coronary repufusion.

References

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Improved Superoxide Dismutase Assay for Clinical Use

To the Editor:

We recently reported a simple method for clinical assay of superoxide dismutase (SOD; EC 1.15.1.1) [1]. We consider this method suitable for samples after ethanol/chloroform treatment (for CuZnSOD assay) or for samples from tissues that contain a trace amount of xanthine oxidase (EC 1.2.3.2) activity. Now, we have modified the method simply by substituting 1 mmol/L diethyleneetriamine-pentaacetic acid (DETAHPAC) for 0.1 mmol/L EDTA and adding 250 μmol/L of disodium bathocuproine disulfonate (BCS), to decrease the interference caused by tissues containing a fairly high concentration of xanthine oxidase, such as liver and small intestinal mucosa (2). Now the reaction mixture for SOD assay contains, per liter, 0.1 mmol of xanthine, 1 mmol of DETAPAC, 50 mg of bovine serum albumin, 25 μmol of nitroblue tetrazolium (NBT), 250 μmol of BCS, about 3 U of xanthine oxidase (Sigma Chemical Co., St. Louis, MO), and 40 mmol of Na₂CO₃ (pH 10.2). For MnSOD assay, 5 mmol/L of NaN₃ is included.

Table 1 shows the interference caused by villus and crypt cells from rat small intestine or mouse liver homogenates in several reaction mixtures. All samples should have an absorbance of 0.0 if no NBT reductase activity is present. To eliminate the interference, we incubated samples with various amounts of protein (20-200 μg) in different reaction mixtures in the absence of xanthine oxidase: (a) old reaction system with EDTA, or (b) DETAPAC instead of EDTA, or (c) DETAPAC plus BCS. After 20 min of incubation at 25 °C, we added 1 mL of 0.8 mmol/L CuCl₂ reagent to terminate the reaction, then read the absorbance at 560 nm. The results indicated that villus cells had the most interference, and the amount of interference was increased as the amount of protein increased. Inclusion of both DETAPAC and BCS in the reaction mixture decreased the interference from samples. The interference is kept within the experimental error if the sample protein in the assay is <50 μg.

To evaluate the sensitivity of this assay after modification, we used as standards pure bovine liver CuZnSOD (Diagnostic Data, Mountain View, CA), Escherichia coli MnSOD from Sigma, and chicken liver MnSOD purified in our laboratory. The results showed that the sensitivity of the modified assay was increased about threefold for CuZnSOD (1 U = 10.5 vs 30 ng), threefold for E. coli MnSOD (1 U = 35 vs 105 ng), and 1.5-fold for chicken liver MnSOD (1 U = 350 vs 500 ng). Therefore, this modified assay will be suitable for use with a variety of tissue samples, with less interference and higher sensitivity.

We used this modified method to measure the total SOD and MnSOD in villus and crypt cells of rat small intestine, a tissue that shows much assay interference. The CuZnSOD activity was obtained by subtracting total SOD from MnSOD. The villus and crypt cells were isolated by the method of Weiser (3). Alkaline phosphatase (EC 3.1.3.1) activity was measured to characterize the villus and crypt fractions (villus: crypt = 267 U per mg of protein:109 U/mg protein, n = 5) (4). We determined protein concentration with the Bradford reagent (Bio-Rad, Richmond, CA). For total SOD, the protein value of the sample was 10-20 μg per tube; we used 8.3 μL of xanthine oxidase per tube to achieve an absorbance at 560 nm of 0.25 in the blank tube. For MnSOD, 20-60 μg of protein and 11 μL of xanthine oxidase were used.

Table 1. Absorbance at 560 nm after 20-Min Incubation of Sample with Various Reaction Mixtures in the Absence of Xanthine Oxidase

<table>
<thead>
<tr>
<th>Protein, μg</th>
<th>EDTA</th>
<th>DETAPAC</th>
<th>DETAPAC + BCS</th>
<th>EDTA + CN</th>
<th>DETAPAC + CN</th>
<th>DETAPAC + BCS + CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.0053</td>
<td>0.0037</td>
<td>0.0027</td>
<td>0.0072</td>
<td>0.0070</td>
<td>0.0050</td>
</tr>
<tr>
<td>50</td>
<td>0.0144</td>
<td>0.0121</td>
<td>0.0100</td>
<td>0.0179</td>
<td>0.0121</td>
<td>0.0102</td>
</tr>
<tr>
<td>100</td>
<td>0.0336</td>
<td>0.0200</td>
<td>0.0143</td>
<td>0.0442</td>
<td>0.0293</td>
<td>0.0210</td>
</tr>
<tr>
<td>200</td>
<td>0.0737</td>
<td>0.0517</td>
<td>0.0251</td>
<td>0.0854</td>
<td>0.0609</td>
<td>0.0440</td>
</tr>
<tr>
<td>Crypt cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.0051</td>
<td>0.0025</td>
<td>0.0016</td>
<td>0.0068</td>
<td>0.0064</td>
<td>0.0058</td>
</tr>
<tr>
<td>50</td>
<td>0.0084</td>
<td>0.0074</td>
<td>0.0056</td>
<td>0.0155</td>
<td>0.0112</td>
<td>0.0105</td>
</tr>
<tr>
<td>100</td>
<td>0.0216</td>
<td>0.0191</td>
<td>0.0123</td>
<td>0.0311</td>
<td>0.0254</td>
<td>0.0226</td>
</tr>
<tr>
<td>200</td>
<td>0.0515</td>
<td>0.0372</td>
<td>0.0176</td>
<td>0.0676</td>
<td>0.0565</td>
<td>0.0428</td>
</tr>
<tr>
<td>Liver cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.0024</td>
<td>0.0006</td>
<td>0.0005</td>
<td>0.0053</td>
<td>0.0044</td>
<td>0.0041</td>
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<tr>
<td>50</td>
<td>0.0079</td>
<td>0.0039</td>
<td>0.0031</td>
<td>0.0128</td>
<td>0.0115</td>
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<tr>
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<td>0.0133</td>
<td>0.0542</td>
<td>0.0468</td>
<td>0.0231</td>
</tr>
</tbody>
</table>
The CuZnSOD activity (U per mg of protein, X ± SEM, n = 5) in villus and crypt cells was 18.4 ± 4.2 and 37.2 ± 8.6, respectively; the MnSOD activity was 8.2 ± 1 and 8.0 ± 1, respectively. Student’s t-test analysis showed that crypt cells contained significantly higher CuZnSOD activity than did the villus cells (P <0.01), but there was no difference in MnSOD activity. Our results are consistent with those obtained from crypt and surface epithelial cells of rat large intestine by Lo- ven et al. (5). The decrease of SOD during differentiation (villus cells are more differentiated than crypt cells) is an exception to the general idea of increasing SOD activity during differ- entiation (6).

References

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Serum Lipoproteins and Coronarographic Features

To the Editor:
Recently (1) it has been stated once again that studies undertaken to correlate plasma lipid concentrations and angiographically documented coronary atherosclerosis diverge in their results and conclusions. Reasons com- monly cited for this are selection of patients, study protocols, analytical methods, and the angiographic tech- niques used. In our opinion, coronary angiographic techniques are a great source of problems.

The ubiquity of this methodology allows one "easily" to obtain a large number of patients, so this type of investigation has become common to verify the correlation between serum lipid concentrations and the presence or progression of atherosclerosis and has taken the place of retrospective or prospective studies, which have proved to be more difficult to manage. But we should keep in mind that, although coronary angiography is a useful diagnostic tool in evaluating coronary stenosis in assessing the need for cardiac surgery, it is used rather uncritically to assess and score atherosclerotic plaques (2, 3).

In fact:
• stenosis can be due not only to atherosclerotic plaques but also to spasms, flogosis, and so on;
• complete stenosis at a single point results in a higher score than do diffuse alterations, which are, however, more important in evaluating atherosclerotic processes;
• progression of stenosis is ascribable to factors other than the athero- genetic ones (i.e., splitting of the plaque and healing), in which case severity of stenosis and serum lipid concentrations could not be closely correlated; and
• the atherosclerotic process can only induce changes in arterial walls without there necessarily being any luminal obstruction.

In addition, atherosclerosis is a systemic process involving the entire body, and the angiographic technique gives us information only about the coronary arteries.

Much work is needed to reach the important goal of clarifying the relation- ship between plasma lipoproteins and the presence of atherosclerosis— e.g., a better comprehension of lipopro-tein metabolism, better analytical pro- cedures, new procedures for "direct quantification of discrete apolipoprotein–defined lipoprotein particles" as outlined by Alaupovic et al. (1)—but clearly we also need a methodology enabling us to identify correctly a patient as having atherosclerosis or not.

Finally, another cause of discrepan- cies probably is underestimated. It is known that LDL lipoproteins with bio-chemical alterations (Acyl-LDL, MDA-LDL, glycated LDL) are not rec-ognized by the LDL receptor and can enter the macrophage without regula- tion, thus creating foam cells (4, 5). These alterations can be present in normocholesterolemic subjects and negatively affect the correlation be- tween serum lipid concentrations and coronaryography results.

References

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A response:

To the Editor:

We thank Graziani et al. for their comments regarding the published re- ports on lipid and apolipoprotein pro- files of patients with coronary artery disease. We agree with their state- ment that angiography can be mis- used as a diagnostic tool. However, there is clear evidence from postmor- tem comparison of angiograms with actual vessel pathology that angiogra- phy can be used to evaluate the degree of atherosclerosis that is present. The first comprehensive study demonstrating this was performed by Dejedar et al. (1). Two additional later studies confirm their findings with computer- ized methods (2, 3). We also agree that atherosclerosis is a systemic process involving many vessels and that angi-