Alterations in Lactate Dehydrogenase Isoenzyme Patterns after Therapy with Streptokinase or Streptococcal Infection

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Serum samples from patients receiving intravenous streptokinase were examined for evidence of interaction in vivo between streptokinase and lactate dehydrogenase (EC 1.1.1.27; LD). We found that this treatment produced a band of LD activity that remained at the electrophoretic origin of LD isoenzyme analysis. Treatment with tissue plasminogen activator produced no such band. The streptokinase–LD complex could be removed from serum by ultracentrifugation. It remained in the circulation for as long as 48 h after streptokinase infusion. A similar phenomenon was observed in a case of pneumococcal sepsis. Examination of supernatants from broth cultures of several species of Gram-positive cocci revealed interactions between human LD and Streptococcus groups A and C and also Streptococcus pneumoniae. Evidently streptokinase can form complexes with LD in vivo after either streptokinase therapy or infection, with consequent alteration of the LD isoenzyme pattern.

We previously found that lactate dehydrogenase (L-lactate:NAD+ oxidoreductase, LD, EC 1.1.1.27) isoenzyme subunit M binds to the thrombolytic agent streptokinase in vitro, presumably because of a structural similarity between LD and plasminogen (1). Formation of complexes between streptokinase and LD in serum typically produces a band (precipitate) of LD activity that remains at the origin in LD isoenzyme electrophoresis.

Intravenous administration of thrombolytic agents such as streptokinase is now standard medical practice for early treatment of acute myocardial infarction (2). However, it is during this early phase of infarction and in subsequent hours that LD isoenzyme analysis is often used to assist in the clinical diagnosis of myocardial injury (3).

Here we present evidence for the in vivo formation of complexes between LD and streptokinase in patients treated with streptokinase. We also report a case of pneumococcal sepsis in which a similar LD electrophoretic anomaly appeared, directly attributable to a bacterial product from that infection. Finally, we have evaluated several bacterial species for production of streptokinase-like substances capable of binding to human LD, which potentially could do so in natural infections. These findings may have further significance in explaining the development of autoantibodies directed against LD.

Materials and Methods

Samples. Blood was sampled into evacuated collection tubes from individuals receiving thrombolytic therapy for acute myocardial infarction. Those patients were treated intravenously with either 1.5 mega-int. units of streptokinase (Streptase; Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876) or 100 mg (58 mega-int. units) of tissue plasminogen activator (TPA; Alteplase recombinant Activase; Genentech, South San Francisco, CA 94080). The sera were available after completion of routine chemistry analyses. Additional samples from nonreperfused patients were chosen from routine LD isoenzyme determinations to include all isoenzymes, for mixing studies and for use as electrophoretic markers.

Bacteria. Clinical isolates of bacteria were identified by standard microbiological methods, propagated on blood agar plates, and inoculated into tryptic soy broth. After incubation for several hours at 37°C, the bacteria culture supernates were cleared of bacteria by centrifugation at 10 000 × g for 2 min.

Methods. LD isoenzyme analysis was performed with the Paragon electrophoresis system, with LD agarose gels, electrophoresis buffer, and enzyme substrate from Beckman Instruments, Inc., Brea, CA 92621.

For high-speed centrifugation we used the Airfuge ultracentrifuge, the A-100 fixed-angle rotor, and 5 × 20 mm cellulose propionate centrifuge tubes (all from Beckman Instruments).

For gel-filtration chromatography we used a 42 × 1.2 cm column of Sepharose CL-2B (Pharmacia, Inc., Piscataway, NJ 08854) equilibrated with phosphate-buffered saline (per liter, 10 mmol of sodium phosphate, pH 7.2, and 149 mmol of sodium chloride). The streptokinase used for gel-filtration studies was pharmaceutical Kabikinase (Smith Kline & French Laboratories, Philadelphia, PA).

Results

Effect of Streptokinase

Streptokinase-induced band of LD precipitate. Multiple serum samples were studied from four patients who received streptokinase within 5 h after initial onset of the chest pain of acute myocardial infarction. Serum was also available from three of these four patients before the onset of symptoms; all showed normal LD isoenzyme distribution (e.g., Figure 1a). A total of 27 samples were collected within 48 h after streptokinase infusion. LD isoenzyme electrophoresis of 16 of these samples showed either a smear of LD activity between LD2 and LD3 (four samples) or a precipitated band of LD activity remaining at the electrophoretic origin (12 samples) (Figure 1b). The normal band for beta-lipoprotein in serum migrated catherolally to the streptokinase-associated LD precipitate (Figure 15). These samples also showed “flipped” patterns (LD1 > LD2) in addition to the early increase of LD1 and LD2 activities characteristic of successful reperfusion of ischemic myocardium.

Serum samples were also obtained from four other patients, who received TPA instead of streptokinase for treatment of acute myocardial infarction. Of 12 such samples collected within 48 h after TPA infusion, none demonstrated smears or precipitated bands of LD activity on isoenzyme electrophoresis.
All serum samples that demonstrated the bands and smears of LD associated with streptokinase administration had been separated from blood clots by centrifugation at about 5000 × g. Subsequent centrifugation at 165 000 × g for 1 h removed the LD precipitate from serum while retaining the same pattern of the other LD isoenzymes (Figure 1, c and d). This finding indicated that the LD precipitate consisted of high-molecular-mass species.

To explore the size of these complexes further, we performed gel filtration by column chromatography on a mixture of nine volumes of serum plus one volume of streptokinase solution (final concentration 15 mega-int. units/L), applying 1 mL of this mixture to a Sepharose CL-2B column as described above. The column was eluted with phosphate-buffered saline, and 1-mL fractions were collected at a flow rate of 25 mL/h. Electrophoresis of each fraction showed that the LD–streptokinase complexes were eluted from the column near the void volume, in fraction 12, whereas the normally migrating LD isoenzymes were eluted in fractions 26 through 32. This result indicated that the complexes had a very high molecular size, greater than the upper limit for fractionation by Sepharose CL-2B (i.e., >4 × 10^7 Da).

Serial patterns of LD isoenzymes after streptokinase infusion. During the 48 h after receiving streptokinase, all four patients initially showed a smear or extra band of LD. The aberrantly migrating LD activity then was absent for several hours, but recurred later, as depicted in Figure 2 for patient 2. Serial measurements on patient 4 revealed shifts in the concentrations of LD4 and LD5 from high to low, coincident with appearance of greater amounts of the precipitate.

Effect of Infection

LD isoenzyme pattern in pneumococcal sepsis. A 74-year-old man had severe pneumonia, with *Streptococcus pneumoniae* being cultured from both sputum and blood. Because he had chest pains, we performed cardiac isoenzyme analysis. By these procedures and electrocardiogram, myocardial infarction was ruled out. The patient demonstrated a band of LD activity at the origin and near absence of LD4 and LD5, although LD isoenzymes 1, 2, and 3 were quite intense compared with a normal pattern (cf. Figure 3, a and b). Supernatant liquid from broth culture of this S. pneumoniae isolate was mixed in equal volumes with normal serum containing all LD isoenzymes. This mixture yielded a pattern of LD isoenzymes (Figure 3c) similar to that of the patient's serum, with a band of LD activity remaining at the origin and near absence of the LD isoenzymes rich in M-subunits. Mixing sterile culture medium with human serum did not have this effect (Figure 3d), and the bacterial supernate alone contained no LD activity (Figure 3e). This suggests that a pneumococcal product also binds to human LD and in fact can do so in vivo during severe infection.

Interactions of LD with products from Gram-positive cocci. To investigate further the spectrum of bacteria that synthesize substances capable of binding to human LD, we examined several species of Gram-positive cocci obtained as clinical isolates from human infections. These bacteria were propagated on blood-agar plates before suspension culture in tryptic soy broth. None of the bacterial culture supernates had LD activity detectable by LD isoenzyme electrophoresis. However, supernates from cultures of *Streptococcus* group A, *Streptococcus* group C, and *S. pneumoniae* contained substances that bound to human LD, with group C producing the greatest amount of binding activity (Figure 4). There was no detectable interaction under these conditions with supernates from *Staphylococcus aureus*, *Staphylococcus epidermidis*, or groups B, D, and F of *Streptococcus*.

Discussion

We have previously shown (1) by in vitro mixing experiments that streptokinase binds to LD isoenzyme subunit M, which shares a small region of homology with the site on plasminogen to which streptokinase is known to bind. The
other LD isoenzyme subunits, H and C (from LD-X, which is expressed solely in spermatozoa), also have regions of homology to this streptokinase binding site, but they differ from LD subunit M and apparently do not bind to streptokinase. The various animal LD-M subunits that do bind to streptokinase have a phenylalanine at amino acid position 162 immediately adjacent to this region of homology, whereas the non-binders have leucine at that position. This region of homology between LD and plasminogen is the presumptive site at which streptokinase binds to LD.

The search for direct interaction between LD and streptokinase was motivated by the observation that some patients treated for acute myocardial infarction with streptokinase subsequently developed autoantibodies against LD within weeks (4, 5). We have hypothesized that the complex of LD with the bacterial antigen streptokinase may stimulate a patient's immune system to produce these autoantibodies and perhaps other as-yet-unrecognized ones as well.

Our object was to look for direct evidence of interactions between LD and streptokinase in the circulation of patients exposed to streptokinase, either through therapeutic administration of that agent or through natural infections with bacteria capable of producing streptokinase or similar substances. We have consistently identified smears and an extra band of LD activity characteristic of LD–streptokinase interactions in the serum of patients who had recently received infusions of streptokinase for acute myocardial infarction. Smears of LD activity or extra bands of LD in the presence of streptokinase represent the same binding phenomenon, but with different concentrations of streptokinase or LD isoenzymes containing M subunits. These aberrant forms of LD were not detected in the serum of patients who received thrombolytic treatment with TPA. This LD–streptokinase complex probably has a high molecular mass (>4 × 10^7 Da), and therefore it does not migrate in an agarose electrophoresis gel optimized for the separation of uncomplexed LD isoenzymes.

One unexpected finding was that these complexes do not simply increase and then decline after streptokinase infusion, but rather can appear, disappear, and then reappear in a patient after a single large dose of streptokinase. These kinetics suggest sequestration either of streptokinase or of complexes, with subsequent release into the circulation. Although the presence or absence of the LD–streptokinase complex or its intensity in patients' samples could be a function of specimen processing (e.g., clot formation, centrifugation, storage), it is not likely, because very vigorous centrifugation was necessary to remove the complexes from serum (Figure 1d). Alternatively, after initial complex formation, there may be reassociation of streptokinase and LD molecules in the circulation or even between deposits of the complexes in tissue and the circulation.

It should be expected that virtually all patients receiving streptokinase infusions will demonstrate atypical smears or bands of LD activity at some time within the first 48 h of treatment. Correct identification of these atypical forms may be useful in excluding the diagnosis of LD autoantibodies (which can show similar patterns) or even suspected technical error in performing the analysis. There may also be selective depletion of the LD isoenzymes rich in M-subunits owing to the participation of those isoenzymes in the complexes that precipitate at the electrophoretic origin.

LD2 and LD3 appear to be less affected by streptokinase than do LD4 and LD5 (Figures 3 and 4) probably because LD4 and LD5 exhibit multiple streptokinase-binding sites (on the M-subunits) that allow them to participate in extensive matrices of complexes with divalent streptokinase molecules. Thus streptokinase infusions for treatment of acute myocardial infarction may diminish the concentration of LD5 in the circulation, which has been used to predict passive congestion of the liver secondary to cardiac infarction and failure (6, 7). This clinical finding, which signals a poorer prognosis after myocardial infarction, might be missed in the diagnosis, owing to interaction of streptokinase with LD5. In addition, other diagnostic information deriving from the ratio of LD1 to the other LD isoenzymes in myocardial infarction (8) may also be altered by streptokinase binding. It does not appear from this study that thrombolytic therapy with TPA is likely to alter the LD isoenzyme patterns by direct binding.

In addition to the occurrence of LD–streptokinase complexes in therapeutic administration, it is also very likely that streptococcal infections can lead to concentrations of streptokinase in the circulation sufficient to alter the electrophoretogram of LD isoenzymes, as seen in our patient with Streptococcus pneumoniae sepsis. Our survey of streptococcal isolates showed that groups A and C had products that reacted with LD. These findings are consistent with early work that established that synthesis of streptokinase is restricted to groups A, C, and G as determined by fibrinolytic assay (9).

This study has shown that, with standard clinical thrombolytic protocols, there is opportunity for complexes between LD and the highly immunogenic streptokinase to form routinely in the circulation. These complexes should be available for processing by phagocytic cells of the immune system in such a way as to stimulate anti-LD antibody production in some patients after intravenous administration of streptokinase. On the other hand, most streptococcal infections of humans probably occur at skin or mucosal surfaces without causing bacteremia. In this regard, it may be noteworthy that a large proportion of anti-LD antibodies cited in the literature have been immunoglobulin A (4, 10–17), whereas other autoantibodies generally are immunoglobulins G or M. This discrepancy may be explained if the original immunogenic stimulus to induction of an anti-LD is at a mucosal surface where the humoral immune response is primarily immunoglobulin A. Therefore, the interaction of streptokinase and like substances with LD at those surfaces may be the initiating event in many of the naturally occurring anti-LD antibodies for which there is no history of streptococcal sepsis or of.
streptokinase infusion.
Whatever the actual route of immune stimulation with streptokinase may be, this report has established that complexes between LD and streptokinase do occur in vivo and are easily recognized on electrophoresis. It also leads us to suggest that the current therapeutic use of streptokinase may lead to increased numbers of anti-LD autoantibodies in the future.

References

Estimation of Reference Intervals for Total Protein in Cerebrospinal Fluid
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Protein in cerebrospinal fluid (CSF) was measured by a modified biuret procedure and in two automated instruments, the Du Pont aca and the Kodak Ektachem. The latter's dry-slide reagent was also evaluated for precision, linearity, and effect of potential interferents. In vitro, ampicillin and vancomycin increase the apparent value for CSF protein as measured with the Ektachem slides. We excluded patients with disorders of the central nervous system, and we estimated the central 95% percentile reference intervals for CSF protein for each of the three methods. We found no age or gender dependence of values. By the biuret procedure, the reference interval is 140 to 620 mg/L.

Additional Keyphrases: method comparison · multilayer film analysis · discrete analyzer · biuret reaction · sex- and age-related effects · antibiotics

Measurement of total protein in cerebrospinal fluid (CSF) is useful in clinical practice. It is a sensitive but nonspecific test, with protein concentrations increasing in inflammatory, neoplastic, traumatic, and other disorders that alter the characteristics of the blood-brain barrier or cause changes in synthesis of immunoglobulin within the central nervous system. Reference intervals in the literature are quite variable, reflecting two problems: the values are method dependent, and the populations used to obtain them are frequently poorly defined. Collecting CSF by lumbar puncture from normal individuals is generally not possible, thus reference intervals for protein in CSF are estimated from values for patients' specimens. Commonly, patients with "neurological disorders" or those with an abnormal myelogram are excluded. A more explicit list of exclusion parameters would be more desirable.

Tibbling et al. (1), using the classical Lowry method, found an age dependence for CSF proteins: older persons had higher values, and the age group of 17 to 40 years had a range of 241 to 485 mg/L. Ahonen et al. (2), using a biuret method, reported a range of 223 to 503 mg/L for 46 patients. Breebaart et al. (3), who used a trichloroacetic acid-Ponceau-S procedure, found a gender- but not an age-dependence in 139 normal persons, their combined range for men and women being 140 to 490 mg/L, with men having slightly higher values. The widely quoted age-dependent reference intervals in Henry (4) are based on "personal experience