Profile of Serum Pregnancy-Associated Plasma Protein A after Sustained Subcutaneous Low Molecular Weight Heparin Administration in Patients with Cerebrovascular Diseases

To the Editor:

Pregnancy-associated plasma protein A (PAPP-A), considered a surrogate marker of increased risk of unstable atherosclerosis and its complications (1, 2), is reversibly bound to the surfaces of several cell types because of heparin’s effective competition for surface binding (3). Heparins, however, especially low molecular weight heparins (LMWH), are routinely used for the treatment and prevention of thromboembolism. Because of the observation that intravenous administration of LMWH and heparin elicits a rapid increase in serum PAPP-A (4), we investigated the time profile of the effect of subcutaneous LMWH administration on PAPP-A concentration in a cerebrovascular disorder population.

Blood samples were collected from 10 patients with ischemic stroke receiving 5000 U of subcutaneous LMWH twice daily (group A) and 10 patients with percutaneous carotid intervention receiving 4375 U heparin intravenously just before stenting (group B). For each patient in group A, the first venous blood sample was drawn after admission. We also collected samples at 3 h after LMWH administration on days 2, 3, and 7, and at 36 h after the last LMWH administration. For group B patients, samples were collected from the arterial sheath just before heparin administration and at 3, 5, 15, 40, 60, and 100 min after administration. The exact time of blood collection was recorded. Serum samples were separated by centrifugation at 1760g at 4 °C for 10 min and stored at −80 °C until analysis. The study protocol was approved by the Medical Ethics Committee of Qilu Hospital, and written informed consent was obtained from each patient. Serum total PAPP-A concentrations were measured with an ultrasensitive ELISA kit with polyclonal anti–PAPP-A antibody (DRG International). The intraassay CVs were 6.9% and 4.3% at 2.7 mU/L and 38.5 mU/L, respectively; interassay CVs were 9.4% and 5.9% at 2.2 mU/L and 36.5 mU/L, respectively.

Both LMWH and heparin administration increased serum PAPP-A concentrations (median, 1.8-fold in group A; median, 4.1-fold in group B). In group A, PAPP-A concentrations increased continuously during the period of LMWH administration (median change, from 12.03 mU/L to 21.80 mU/L; P = 0.002). This increase was followed by a normalization of the concentration after the drug was discontinued (Fig. 1). In group B, the PAPP-A concentration peaked within 5 min (median, 51.56 mU/L), remained increased for 15 min (median, 43.75 mU/L), and then decreased to 16.4 mU/L at 100 min. The response in group A was lower (peak median concentration, 21.80 mU/L vs 51.56 mU/L; P < 0.0001) but more sustained than in group B, possibly because of the differences of medication, dose, and administration patterns.

To document the relationship between sustained subcutaneous LMWH administration and serum PAPP-A concentration more clearly, we also measured the serum LMWH concentration by high-performance capillary electrophoresis. After subcutaneous injection twice daily for about 1 week, the LMWH concentration gradually increased (median, from 370 U/L on day 2 to 650 U/L on day 7; Fig. 1), which may have contributed to the gradually increasing PAPP-A concentration.

Because the LMWH concentration showed patient-to-patient variation (Fig. 1), we could not investigate whether the extent of heparin-induced PAPP-A release correlated with the atherosclerotic lesions of the vasculature. Further-

1 Nonstandard abbreviations: PAPP-A, pregnancy-associated plasma protein A; LMWH, low molecular weight heparin.
more, we found no significant correlations between the PAPP-A concentration after LMWH and heparin administration and age (P = 0.759 and 0.166, respectively). The PAPP-A concentration decreased slightly on the third day of the first part of the observation period, but the cause remains to be investigated. Although the PAPP-A response caused by heparins is clear, the origin of the increased PAPP-A is not. In addition to its role in the development of atherosclerotic lesions in animals, serum PAPP-A has also been reported as an indicator of the peripheral atherosclerotic burden in elderly patients (5). Because the increase in PAPP-A occurred within a few minutes after intravenous heparin injection, PAPP-A might be released from the vasculature with atheromatous plaques. Free PAPP-A is the major form of the PAPP-A increasing in the circulation (4), but only the surface-bound PAPP-A is active (3). Heparins could induce PAPP-A release from the cell surface and into the blood circulation. Therefore, heparins might be valuable in therapy for unstable atheromatous plaques.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Role of Sponsor: The funding organizations played a direct role in the design of the study, choice of enrolled patients, review and interpretation of data, and preparation and final approval of the manuscript.

Acknowledgments: We thank the Department of Neurology of Qilu Hospital for their help in sample collection.

References

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Previously published online at DOI: 10.1373/clinchem.2010.152702

Detection and Elimination of Interference by the Heterophilic Antibody in Antibody Microarray–Based Immunoassay

To the Editor:

Antibody microarray–based multiplex immunoassays can be applied in the early and differential diagnosis, staging, and prognosis of disease (1). In addition to the technical challenges that are unique to microarrays, this approach also faces the same problems as other immunoassays, such as interference by heterophilic antibodies, when it is used in clinical applications.

Heterophilic antibodies are antibody-binding substances in the serum that can interfere with 2-site immunoassays involving animal antibodies and lead to erroneous analyte quantification (2). Various methods have been proposed to alert laboratories to the presence of or to block the effects of these interfering substances (3, 4). Antibody microarrays are often 2-site immunoassays configured with antibody pairs, with the 2 antibodies being of mouse origin, rather than a mouse monoclonal antibody and a polyclonal antibody from another species. With 2 monoclonal antibodies in a single system, the potential for interference increases substantially. Because of the diversity of heterophilic antibodies and the difference in the capture antibody in microarrays, none of the current countermeasures can completely eliminate interference by heterophilic antibodies.

To take advantage of the capability of protein microarrays to produce vast quantities of information and to facilitate cross-referencing among data points (5), we designed a new way to identify heterophilic interference that is simple, inexpensive, and effective.