Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 850 (2007) 586–587
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Letter to the Editor

Asymmetric dimethylarginine—comparison of HPLC and ELISA methods

Keywords: ADMA; ELISA; HPLC

The adverse effect of hyperhomocysteinemia on a vascular wall can be partially explained by an increasing plasma concentration of asymmetric dimethylarginine (ADMA), a potent inhibitor of nitric oxide synthase. Two L-arginine derivatives: N^G-monomethyl-L-arginine (L-NMMA) and N^G,N^G-dimethyl-L-arginine (asymmetric dimethylarginine – ADMA) competitively block the nitric oxide synthase (NOS) active site and then selectively decrease NO synthesis. As the ADMA blood concentration is about ten-fold higher than that of L-NMMA, it is considered to be the predominant endogenous NOS inhibitor [1,2].

Patients with end-stage renal disease face a particularly high risk of a cardiovascular disease and possible cardiovascular mortality. Part of their increased risk is due to a higher prevalence of the established risk factors, such as arterial hypertension, diabetes mellitus, lipid metabolism disorders and anemia. Inflammatory processes, high sympathetic activity, and the accumulation of an endogenous inhibitor of NOS – asymmetric dimethylarginine (ADMA), have recently emerged as the cardiovascular risk factors of paramount importance [3]. The time-consuming and complicated chromatographic method (HPLC with previous derivatization) is needed for the measurement of ADMA levels. The HPLC methods of determining ADMA concentration in plasma applying different detection techniques have already been published [4–9] and only recently has an immunochemical method taken place based on ELISA technique [10].

We collected 80 EDTA plasma samples, blood was collected after an over-night (12 h) fasting (40 healthy blood donors and 40 hemodialysis patients) and then compared the results of ADMA concentrations, obtained by HPLC and ELISA methods. The sampling procedure was standardized, plasma samples were collected in EDTA tubes and centrifuged at $3600 \times g$ for 5 min within 30 min; plasma was separated and stored frozen at −70 °C until analysis. For the HPLC method, we used equipment from Thermo separation product (Miami, Florida, USA). After the solid-phase extraction on a polymer cation-exchange column (OASIS MCX, Waters, Milford, MA, USA) and the following derivatization with *o*-phthaldialdehyde, ADMA and its stereoisomer, symmetric dimethylarginine (SDMA), were separated simultaneously within 30 min using C18 column (Waters, Milford, MA, USA; mobile phase 8.7% acetonitrile, 50 mmol/l phosphate buffer, pH 6.5) with fluorescence detection (excitation 340 nm, emission 450 nm). N^G-monomethyl-L-arginine was used as an internal standard. Retention times for ADMA and SDMA are 17.4 and 18.4 min, respectively. This technique is similar to the method described by Teerlink et al. [9] with minor modifications. Using a 0.2 ml sample volume, linear calibration was obtained and the signals of stable derivates (ADMA and SDMA) were near the baseline resolution. Although we did not use derivatization just before analysis, our recovery of ADMA was good (92%); also the reproducibility (5.2%) and the detection limit (below 0.13 μmol/l) were similar to those described by Valtonen et al. [11].

For ADMA immunochemical quantification, the ELISA method (kit ADMA® ELISA, DLD Diagnostika GmbH, Hamburg, Germany) and the AUTO-EIA II microplate reader (Labsystems Oy, Espoo, Finland) were used. This competitive method uses the microtiter plate format. ADMA is bound to the solid phase and ADMA in samples is acylated and competes with the bound ADMA for a fixed number of rabbit anti-ADMA antiserum binding sites. After the equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The bound antibody is detected by anti-rabbit peroxidase and TMB (3.3′,5.5′-tetramethylbenzidine) as a peroxidase substrate. The final product of this reaction is monitored at 450 nm. The amount of antibody is inversely proportional to the ADMA concentration in the sample.

The analytical sensitivity of the ELISA method, expressed as a detection limit, was 0.05 μmol/l; this corresponds to the kit manufacturer's data. The inter-assay CV of ADMA for the kit control 1 (ADMA concentration 0.510 ± 0.086 μmol/l) was 14.0% and for the kit control 2 (0.892 ± 0.162 μmol/l) 18.0% ($n = 5$). Both kit controls fell between the target range (control 1: 0.24–0.58 μmol/l, control 2: 0.6–1.0 μmol/l). The repeatability of the ELISA method, expressed as an intra-assay coefficient

1570-0232/$ – see front matter
doi:10.1016/j.jchromb.2006.12.009

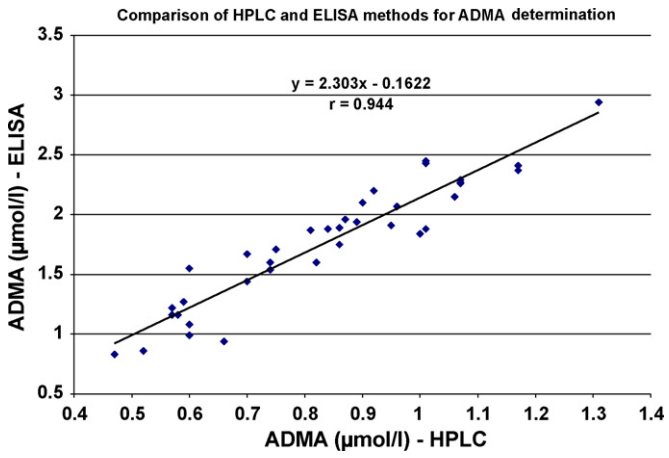

Fig. 1. Comparison of HPLC and ELISA methods (80 different samples).

of variation and calculated from 20 measurements of plasma pool (mean ADMA concentration 1.58 μmol/l), was 11.8%. We also measured in duplicate 40 different plasma samples of hyperhomocysteinemic patients (mean ADMA concentration 1.59 μmol/l, range from 0.82 to 2.49 μmol/l) and expressed the repeatability from the differences between the parallel measurements; the variation coefficient was 4.75%.

We compared HPLC and ELISA methods for ADMA determination in a wider range than Valtonen et al. [11]. The ADMA concentration range of our samples was 0.3–3.0 μmol/l. In spite of differing principles, both methods showed a very good correlation ($r = 0.944$, $p < 0.0001$), Fig. 1. These results show that the time-consuming HPLC method for ADMA determination can be replaced by ELISA which gives comparable results, has good reproducibility and is more suitable for greater series of samples.

Conflicting results were published in studies by Schultze et al. [10] and Valtonen et al. [11]. In our study, we confirmed Schultze's results; our correlation coefficient was only a little lower ($r = 0.944$, $p < 0.0001$ versus $r = 0.991$ obtained by Schultze et al.). Schultze used the same ELISA method but employed a different chromatographic technique (GC–MS) and also the range of our results was different. As Valtonen et al. [11] explained there were some points which can change a correlation curve. Firstly plasma samples were remarkably low in Schultze et al. [10]. On the other hand we measured ADMA concentration with a wide range. Secondly, the kits from the above mentioned papers did not have the same lot number. The ELISA method, although it is rather less precise, gives comparable results with those obtained by HPLC as can be seen from the Fig. 1.

According our results, the ELISA method is more accessible for laboratories without specific instruments (LC, GC–MS, OASIS columns, etc.) and especially for a larger sample series.

Acknowledgement

This study was supported by the Grant Agency of Charles University in Prague No. 78/2005/C/LFP. University in Prague No. 78/2005/C/LFP and Research Project MSM 0021620819.

References

[1] J.T. Kielstein, J.C. Frölich, H. Haller, D. Fliser, Nephrol. Dial. Transplant. 16 (2001) 1742.
[2] H. Miyazaki, H. Matsuoka, J. Cooke, U. Michiaki, S. Ueda, S. Okuda, Circulation 99 (1999) 1141.
[3] C. Zoccali, F. Mallamaci, G. Tripep, Kidney Int. 85 (2003) 105.
[4] J. Martens-Lobenhoffer, O. Krug, S.M. Bode-Boger, J. Mass Spectrom. 39 (2004) 1287.
[5] L.F. Huang, F.Q. Guo, Y.Z. Liang, B.Y. Li, B.M. Cheng, Anal. Bioanal. Chem. 380 (2004) 643.
[6] J. Albsmeier, E. Schwedhelm, F. Schulze, M. Kastner, R.H. Boger, J. Chromatogr. B 809 (2004) 59.
[7] D. Tsikas, B. Schubert, F.M. Gutzki, J. Sandmann, J.C. Frolich, J. Chromatogr. B 798 (2003) 87.
[8] E. Causse, N. Siri, J.F. Arnal, C. Bayle, P. Malatray, P. Valdiguie, R.J. Salvayre, J. Chromatogr. B 741 (2000) 77.
[9] T. Teerlink, R.J. Nijveldt, S. de Jong, P.A. van Leeuwen, Anal. Biochem. 303 (2002) 131.
[10] F. Schultze, R. Wiseman, E. Schwedhelm, K. Sydow, J. Albsmeier, J.P. Cooke, et al., Clin. Chem. Lab. Med. 42 (2004) 1377.
[11] P. Valtonen, J. Karppi, K. Nyyssonen, V.P. Valkonen, T. Halonen, K. Punnonen, J. Chromatogr. B 828 (2005) 97.

R. Široká*
L. Trefil
D. Rajdl
J. Racek
R. Cibulka
Institute of Clinical Biochemistry and Hematology, Charles University, Medical Faculty Pilsen, Alej Svobody 80, 304 60 Plzeň, Czech Republic

* Corresponding author. Tel.: +420 377104234; fax: +420 377104234.
E-mail address: sirokar@fnplzen.cz (R. Široká)

17 August 2006

Available online 2 January 2007