Clinica Chimica Acta 493 (2019) 20–24

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Plasma bradykinin concentrations during septic shock determined by a novel LC-MS/MS assay

Mikael Lindström[a,*,1], Miia Valkonen[b,1], Niina Tohmola[a], Risto Renkonen[a,c], Tomas Strandin[c], Antti Vaheri[c], Outi Itkonen[a]

[a] HUSLAB, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[b] Division of Intensive Care Medicine, Department of Anesthesiology, Intensive Care and Pain Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[c] Faculty of Medicine, University of Helsinki, Finland

ARTICLE INFO

Keywords:
Bradykinin
Sepsis
Septic shock
LC-MS
Vascular permeability

ABSTRACT

Background: Bradykinin is an important mediator of inflammation and vascular permeability and could have an important role in the development of septic shock. Measurement of bradykinin by immunological methods may suffer from interference and lack of specificity. We developed and validated a liquid chromatography mass spectrometry assay (LC-MS/MS) for plasma bradykinin.

Methods: We used plasma samples from healthy volunteers (n = 19) and patients with septic shock (n = 47). Stable isotope bradykinin internal standard was added to samples before solid-phase extraction and quantification by LC-MS/MS. Stability of bradykinin was studied for 12 months.

Results: Our assay has good sensitivity (0.1 nmol/l) and a wide linear range (0.1–1000 nmol/l). Bradykinin added to plasma was stable for 12 months at −20 °C when a mixture of protease inhibitors was added at sampling but degraded during repeated freezing and thawing. Bradykinin concentration in plasma from septic shock patients (< 0.1–0.6 nmol/l) did not change significantly during shock and recovery but differed slightly from that in healthy individuals (0.5–1.1 nmol/l).

Conclusions: Our bradykinin assay was successfully used to determine bradykinin concentrations in plasma samples. Intensive care unit patients with septic shock had low concentrations of plasma bradykinin during both shock and recovery phases.

1. Introduction

Definition of sepsis has been recently updated as a “life threatening organ dysfunction due to a dysregulated host response to infection” [1]. Septic shock is a subset of sepsis with high mortality. The pathophysiology leading to organ failure is characterized by tissue hypoperfusion due to vasodilatation, hypotension, microvascular thrombosis and myocardial depression. In addition, inflammation causes endothelial dysfunction, loss of endothelial barrier leading to capillary leakage and edema. Impaired respiratory chain function, due to oxidative damage to mitochondria further contributes to energy deprivation in tissues [2].

Bradykinin (BK) is among the effectors causing increased vascular permeability and capillary leakage. It is a nonapeptide (RPPGFSPFR) produced by proteolytic cleavage by kallikrein from high molecular weight kininogen (HMWK) and has been found to contribute to several inflammatory processes and hereditary angioedema (HAE). The presence of bradykinin further stimulates the release of known potent vasodilators such as nitric oxide from the endothelium *via* two G-protein coupled receptors (BK1 and BK2) [3,4]. The effects of excess bradykinin on endothelial cells can be blocked by bradykinin-2-receptor antagonist, icatibant, currently used for the treatment of HAE. However, during septic shock the contribution of bradykinin on capillary leakage and vasodilatation still needs to be established. We were prompted to study bradykinin in septic shock since patients with very severe hantavirus infection manifested by capillary leakage syndrome and shock were recently suggested to benefit from icatibant [5–7].

Analysis of bradykinin is challenging due to the very low concentration present in circulation. In addition, the nonapeptide is rapidly

Abbreviations: LC-MS/MS, liquid chromatography tandem mass spectrometry; SPE, solid-phase extraction

* Corresponding author.
E-mail address: mikael.lindstrom@hus.fi (M. Lindström).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cca.2019.02.023
Received 8 November 2018; Received in revised form 21 February 2019; Accepted 21 February 2019
Available online 22 February 2019
0009-8981/

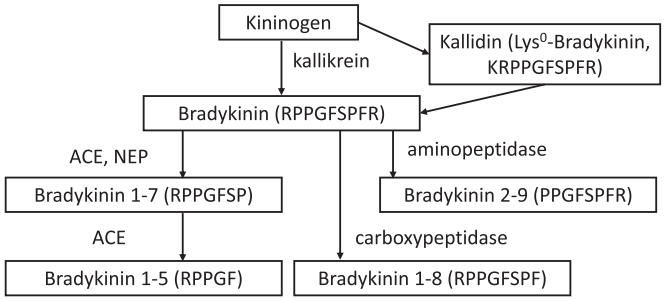

Fig. 1. Bradykinin formation and enzymatic metabolism products according to Campbell et al. [1]. ACE, angiotensin converting enzyme; NEP, neutral endopeptidase.

degraded *in vivo* [8]. Bradykinin can also form *ex vivo* during sampling and storage due to enzymatic processes unless protease inhibitors, such as angiotensin converting enzyme (ACE) inhibitors, are used to prevent this (Fig. 1). Due to the pre-analytical challenges of preserving the intact peptide, some laboratories have instead opted to assay bradykinin metabolites such as bradykinin 1-5 (BK(1-5)), a pentapeptide fragment of bradykinin, which is considered more stable [9]. Another issue encountered with immunochemical assays is that the antibodies used may exhibit some cross-reactivity for the precursors and metabolites of bradykinin [10,11].

In this paper we present an analytically and clinically validated liquid chromatography tandem mass spectrometry (LC–MS/MS) assay for determination of plasma bradykinin. We determined bradykinin concentrations in samples from patients with septic shock treated in intensive care unit (ICU) in Helsinki University Central Hospital. Multiple samples were collected from each patient to detect changes in plasma bradykinin concentration from shock to recovery.

2. Materials and methods

2.1. Reagents and materials

An inhibitor mix containing aprotinin (10,000 KIU/ml), trypsin inhibitor (800 µg/l), hexadimethrin bromide (4 mg/ml), 1,10-phenanthrolin (10 mg/ml) and EDTA-K2 (20 mg/ml) was prepared in water and stored aliquoted at −20 °C [12]. Stock solutions of 5 µmol/l (5.3 mg/l) bradykinin, BK(1–5) (2.8 mg/l) and corresponding stable isotope-labeled internal standards (IS; $^{13}C_9$,^{15}N for BK, ^{13}C,^{15}N for BK (1-5)) were prepared in a mixture of albumin solution (10 mg/l)/inhibitor mix (9/1, vol/vol). All bradykinin peptides were from Innovagen AB, Lund, Sweden. Bradykinin calibrators (0.1–1000 nmol/l) and IS working solution (100 nmol/l) in water were freshly prepared from the stock solutions. Sample pretreatment was performed employing 96-well Oasis WCX µElution plates (Waters, Milford, MA, USA). MS-grade methanol, acetonitrile (ACN), formic acid, acetic acid and ammonium hydroxide were from Fluka (Sigma-Aldrich Co.). Trifluoracetic acid was from Thermo Scientific. All reagents were of the highest analytical grade.

2.2. Samples and patients

Blood samples (5.5 ml) were drawn into Monovette syringes (Sarstedt, Nümbrecht, Germany) containing 500 µl of the inhibitor mix. Samples were placed on ice for 60 min, and then centrifuged for 10 min at 3000 ×*g* in 4 °C. Samples from healthy volunteers were collected during 2015–2016 from laboratory staff (16 women and 3 men) with a median age of 48 yrs. (range 30–63 yrs). The samples were kept at −20 °C until analysis unless otherwise stated. Patient samples were collected from 47 patients with septic shock (28 men) with a median age of 58 yrs. (range 33–82 yrs) and kept at −80 °C until analyzed.

Table 1
Characteristics of the patients. The patients included in the study (n = 47) had septic shock according to its third (2016) definition (1): sepsis with persisting hypotension requiring vasopressors to maintain mean arterial pressure over 65 mmHg and serum lactate level > 2 mmol/l as a sign for tissue hypoperfusion, despite adequate volume resuscitation.

Characteristic	Median (range)
M/F	28/19
Age, yrs	58 (33–82)
LOS[a], days	11.0 (3–47)
MPFB[b], liters	6.3 (0–21.3)
Hospital mortality	23%
CRRT[c]	51%
Norepinephrine dose, ug/kg/min	0.6 (0.1–1.6)
Infection	
Blood culture positive	45%
Gram negative infection identified or likely due to focus	40%

[a] Length of stay in ICU.
[b] Maximum positive fluid balance in liters in ICU during sampling period 1 (shock).
[c] Continuous renal replacement therapy during sampling period 1 (shock).

For quality assurance (QA), we employed healthy donor plasma spiked with 0.5, 9, 50 and 400 nmol/l bradykinin.

The study was approved with a waiver of informed consent by the local ethical committee. An informed consent was obtained from all healthy individuals and from 47 patients of the 53 screened. Adult patients who fulfilled the 2016 definition of septic shock were included in the study (1). Both community acquired or hospital related infections were included. The patients were treated following the surviving sepsis guidelines including appropriate and timely antimicrobial therapy and source control. Most patients developed multiple organ failure and required fluid replacement, vasoactive agents, mechanical ventilation and continuous renal replacement therapy (CRRT)(Table 1).

Due to previous studies showing wide interindividual variation in plasma bradykinin concentrations, six samples for bradykinin determination were collected from each patient. Bradykinin concentrations were thus followed individually from shock phase to recovery (Table 2). Three samples (with 8 h intervals) were collected both during the shock phase as well as the recovery phase. The patient was considered to enter the recovery phase when there were no more signs of tissue hypoperfusion and no more vasoactive drugs.

2.3. Sample and calibrator preparation

Calibrators, QA samples and plasma samples (500 µl) were mixed with 100 µl of IS working solution and transferred to a WCX µElution plate pre-wetted with 500 µl of methanol and 500 µl water. The plate was washed with 500 µl of 40% (v/v) ACN and 500 µl 5% (vol/vol) ammonium hydroxide. Bradykinin was then eluted with 100 µl of ACN/water/trifluoracetic acid (75/24/1, vol/vol), dried under in nitrogen flow, and re-suspended into HPLC eluent (ACN/90 mmol/l ammonium formate, 10/90, vol/vol).

Table 2
Sampling time points for the ICU patients.

Sample number	Sampling time
1	ICU patient screened and fulfilled criteria of septic shock [1]
2	8 h after the first sample
3	16 h after the first sample
4	Noradrenaline administration stopped
5	8 h after stopping noradrenaline administration
6	16 h after stopping noradrenaline administration

2.4. LC–MS/MS

Our instrument setup comprised an Agilent 1200 liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) and a TQ 5500 triple quadrupole mass spectrometer (AB Sciex, Toronto, Canada) equipped with a Turbo-V electrospray ion (ESI) source. Chromatographic separation was achieved using Kinetex Biphenyl column (50 × 2.10 mm, 2.6 µm, Phenomenex) operated at 40 °C. Mobile phase A was ACN and mobile phase B 90 mmol/l ammonium formate, pH 4. For gradient, A was kept at 10% for 1 min, then ramped to 98% in 6 min, kept at 98% for 2 min, then ramped back to 10% in 1 min and kept at 10% for 3 min. The flow rate was 400 µl/min. For MS/MS detection of bradykinin we followed the transitions of *m/z* 177 → 115 and *m/z* 181 → 164.2 for bradykinin and IS, respectively. While not quantitated, bradykinin metabolite BK(1–5) was monitored (transitions *m/z* 573.5 → 417.1 for BK(1–5) and 583.4 → 427.3 for BK (1–5) IS) to observe whether the bradykinin in the samples was degraded, *i.e.* if BK(1–5) was present. MS instrument settings for curtain, nebulizer and heater gasses were 20, 55 and 45 l/min, and collision gas setting at 8. Data was collected by the Analyst software (Version 1.6.2, AB Sciex).

2.5. Analytical validation of the method

The linearity was determined by preparing and analyzing 15 calibrator dilutions (0.025–1000 nmol/l) on three different days. The calibration curve was derived using $1/x^2$ weighted linear least-squares regression. The limit of detection (LOD) was determined as the lowest concentration with a signal to noise ratio of 3. Relative error (RE) and the coefficient of variation (CV) were calculated. Limit of quantification (LOQ) and linear range was determined as the lowest concentration and range, respectively, that could be measured with RE and CV < 20%. Intra- and inter-assay variation were calculated from the QA sample results in a single analysis (n = 12) and on 14 separate days, respectively. Accuracy of added bradykinin was determined using three plasma samples (endogenous bradykinin concentration 0.8–1.0 nmol/l) with and without bradykinin spike of 0.5, 9, 100 and 300 nmol/l. Matrix effect was studied by extracting two plasma samples in triplicate, spiking with 200 nmol/l of bradykinin after sample extraction and comparing with spiked elution buffer. Effect (attenuation or enhancement) on the measured signal was calculated from the peak areas.

2.6. Preanalytical validation

We studied sample stability at −20 °C using fresh plasma samples from healthy individuals (n = 15) spiked with 100 nmol/l bradykinin. The sample aliquots were analyzed on days 0–7 (daily), 28, 56, 77, 98, 140, 260 and 360. The effect of repeated freezing and thawing from the same aliquot was studied by analysis at 1 week intervals until bradykinin decomposition. Samples with < 20% change from initial concentration were considered stable.

2.7. Statistical methods

We used Analyse-it for Microsoft Excel 2016 (Ver. 4, Analyse-it software Ltd., http://www.analyse-it.com) to run Deming regression and paired t-tests. A p-value < 0.05 was considered statistically significant. A value of 0.05 nmol/l was used for patient sample results < LOQ.

3. Results

3.1. Analytical validation of the bradykinin assay

The retention time of bradykinin and IS was 5.9 min (Fig. 2). The method was linear over the concentration range 0.1–1000 nmol/l

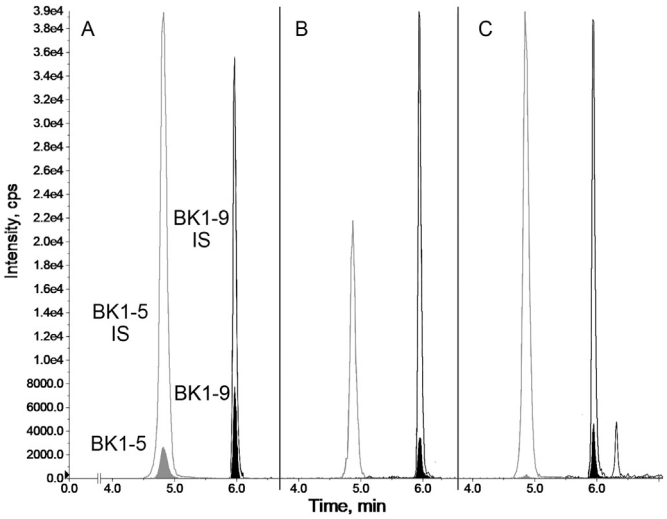

Fig. 2. Chromatograms of bradykinin 1-5 (BK1-5) and bradykinin (BK1-9) calibrator, containing 10 and 1 nmol/l of respective analyte (A), QA sample containing 0.5 nmol/l bradykinin (BK1-9) (B) and a patient sample containing 0.4 nmol/l bradykinin (BK1-9).

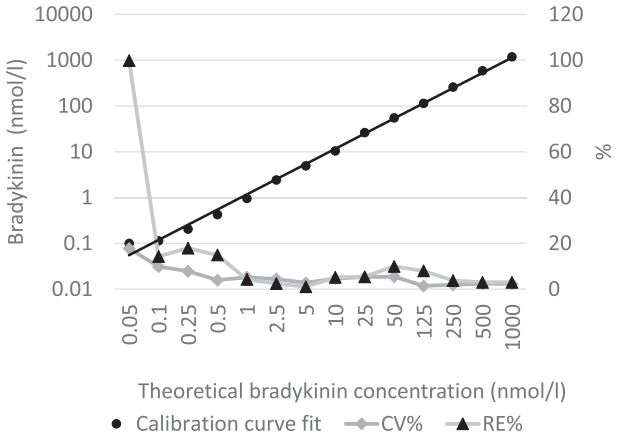

Fig. 3. Linear range (R^2 = 0.9959) and accuracy (CV and relative error) of the bradykinin assay.

(Fig. 3), the LOD was 0.03 nmol/l and LOQ 0.1 nmol/l. After analytical validation we used 0.1–10 (0.1, 0.5, 1, 5 and 10) nmol/l calibrators only. The intra-assay CVs were 5.6%, 3.6% and 3.2% and the inter-assay CVs 8.8%, 7.6%, 6.5% and 4.6% at 0.5, 9, 50 and 400 nmol/l bradykinin, respectively. The mean accuracy of bradykinin (0.5, 9, 100 and 300 nmol/l) spiked into plasma samples (n = 3) containing 0.8–1.0 nmol/l endogenous bradykinin were 97% (range 93–110%), 88% (range 83–92%), 82% (range 79–87%) and 80% (range 73–84%), respectively. Plasma matrix attenuates MS-signal by 50%.

3.2. Preanalytical validation

Bradykinin (100 nmol/l) spiked into plasma containing the protease inhibitor mix was stable for one year at −20 °C. However, bradykinin is not stable during repeated freezing and thawing. After the first thawing bradykinin concentration was 81% of the initial concentration and after the second freezing and thawing cycle it was only 55%.

3.3. Bradykinin in plasma samples

Bradykinin concentration was below the assay LOQ of 0.1 nmol/l in 137 (59%) out of 248 patient samples (Fig. 4). In the remaining samples

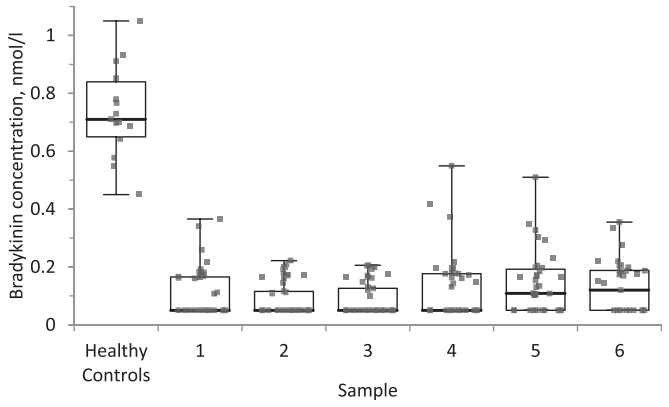

Fig. 4. Bradykinin concentrations in 248 samples from ICU patients (n = 47) at different sampling points. Squares represent the individual samples, while the black line is the median concentration. We used value 0.05 nmol/l for concentrations < 0.1 nmol/l.

the median concentration was 0.2 nmol/l (range of 0.1–0.6 nmol/l). Although most patients (80%) showed no difference in plasma bradykinin concentrations between the shock and recovery phases, three had slightly elevated plasma bradykinin on admission only (shock phase), while seven had an increase during the recovery phase (sampling points 4–6). The median bradykinin concentration was 0.7 nmol/l (range 0.5–1.1 nmol/l) in the samples from healthy volunteers (n = 15), which is higher ($p < 0.0001$) than that in the patient samples.

4. Discussion

We have developed and validated a new LC-MS/MS assay for plasma bradykinin. Our assay is sensitive (LOQ 0.1 nmol/l) and has a broad linear range (0.1–1000 nmol/l), but due to very low patient bradykinin concentrations, only 0.1–10 nmol/l calibrators were used for clinical validation. The reproducibility is good, with both intra- and inter-assay CVs below 10%. Analytically, our assay compares favorably to the previous ones based on LC-MS/MS with LOQs of 10 nmol/l or higher [13,14].

In humans, bradykinin is formed from HMWK by plasma kallikrein (Fig. 1) [15]. In tissues, tissue kallikrein generates kallidin (Lys^0-BK), which can be converted into bradykinin by aminopeptidase-mediated enzymatic cleavage. Several enzymes, such as ACE, neutral endopeptidase and caxboxypeptidase can further cleave bradykinin into BK(1-5), BK(1-7) and BK(1-8), respectively. While a low detection limit of few pmol/l has been claimed for an immunoassay in a previous study [16], the assay may lack specificity and suffer from cross-reactions caused by bradykinin precursors, degradation products, other kinins and their metabolites. MS/MS based detection has superior specificity towards the bradykinin molecule.

Bradykinin concentrations assessed in this study (< 0.1–1.1 nmol/l, n = 264) were overall lower than those (< 10–162 ng/ml or < 10–153 nmol/l) reported by van den Broek et al. [14] using an LC-MS/MS assay and samples from breast cancer patients (n = 6) and healthy controls (n = 6). In their limited study, serum samples were used after a clotting time of 30 mins at room temperature. Thus, the measured bradykinin concentrations are likely to be affected by both *ex vivo* bradykinin production and rapid degradation to an unknown extent. We did not observe detectable concentrations of BK(1–5) in our samples, whilst Seip et al. [17], using LC-MS/MS, reported an increase in BK(1–5) during a HAE attack. In their study, Seip et al. [17] used an indirect assay for estimating bradykinin by quantitating BK(1-5). The amount of BK(1-5) was below 35 pmol/l (detection limit) in healthy donor serum separated at room temperature, while patients showed concentrations of up to 1.7 nmol/l.

Our LC-MS/MS assay employs a similar sample pretreatment by SPE as that by van den Broek et al.. Published radioimmunoassays (RIAs) [10–12,16,18] predominantly use organic solvent precipitation and extraction of the samples. Earlier reports [18] without enzyme inhibitors have reported blood kinin concentrations of up to 460 ng/ml, while following studies with different inhibitor mixes [10,12,16] claim bradykinin concentrations of 0.2–7 pmol/l in healthy controls. The differences in sample preparation may explain the difference between the RIA results of Nussberger et al. (0.2–7.1 pmol/l) [10] and our LC-MS/MS assay (0.6–1.1 nmol/l), because the inhibitor mix used was largely the same in both studies. Additionally, precipitation may lead to loss of peptides due to the nonselective nature of the method. In our study the use inhibitor mix in the sampling syringes and immediate cooling of the samples are likely to prevent both *ex vivo* production and degradation of bradykinin after sampling. This is supported by the absence of BK(1–5) in our samples.

We found that bradykinin was stable in human plasma when frozen with the inhibitor mix but not during repeated freezing and thawing. The inhibitor mix used consists of reversible protease inhibitors. The samples were allowed to reach room temperature during repeated freezing stability testing. It is thus possible, that chosen inhibitor mix may only work, when kept at ≤4 °C. To overcome this issue of instability, it is necessary to keep the blood samples chilled at ≤4 °C during preparation. This meant drawing the blood into pre-chilled syringes, placing the syringes on ice and centrifuging them at 4 °C, and finally aliquoting the fresh plasma samples prior to freezing. Van den Broek et al. observed a 30–60 min half-life for spiked bradykinin in bovine plasma and human serum when no inhibitors were used [14], which is similar to the findings by Heudi et al., who report a value of 40 min for untreated human plasma. [19]. Seip et al. reported an up to 60% increase in BK(1–5) degradation fragment concentration in four hours, when blood samples (with added EDTA) were left at room temperature or repeatedly frozen and thawed [17]. With the inhibitor mix chosen for this study, we found that bradykinin in plasma samples was stable for at least 3 h from drawing the blood. We found that plasma bradykinin was stable for one year at −20 °C. To our knowledge, earlier studies have not assessed the long-term stability of bradykinin.

In our study bradykinin concentrations measured in samples from patients with septic shock were very low or below the LOQ. A previously published porcine model for septic shock using an LC-MS/MS method reported similar findings despite a massive capillary leakage observed in the animals [20]. There are several possible reasons for the low concentrations of plasma bradykinin found in this study. Bradykinin may be locally released and effectively bound to its G-protein coupled B2R receptor on the surface of endothelial cells thus mediating capillary leakage and the concentrations in circulation may not reflect the bradykinin in action. The half-life of bradykinin in human serum is estimated to be < 30 s [21]. Large percentage (51%) of these septic shock patients received CRRT during the sampling periods. CRRT may remove small molecules like bradykinin from the circulation. Furthermore, plasma of the patients may be diluted by fluid replacement and vascular leakage. To support this, we have also observed, that plasma HMWK concentration in acute phase is lower than that in recovery phase (unpublished data). In addition, our mixed-ICU patients had several different infection focuses as well (Table 1).

Previously, bradykinin-2 –receptor antagonist deltibant led to reduced mortality in human cases with pure gram-negative etiology [22] and severe hantavirus infection for septic shock suggesting bradykinin involvement in these conditions. However, we did not find higher bradykinin concentrations in our patients with proven or suspected gram-negative origin for septic shock (Table 1). Instead, we found that bradykinin concentration in samples from healthy individuals was higher than that in patient samples ($p < 0.0001$), although still in the low nanomolar range. Thus, determination of plasma bradykinin in septic shock patients cannot be suggested to support the definition of use of bradykinin-2 receptor antagonists.

In conclusion, our newly developed LC-MS/MS assay is well suited for research purposes. Although the majority of patient plasma bradykinin concentrations turned out too low to quantify accurately, healthy individual plasma concentrations were within the linear measurement range. The protease inhibitor mix preserved the samples when frozen for at least 12 months. The developed assay can potentially be utilized for studying other bradykinin mediated medical conditions in the future.

Funding

This work was partly supported by Sigrid Jusélius Foundation and by Magnus Ehrnrooth Foundation.

Disclosures

The authors declare no conflicts of interest regarding this article.

Acknowledgements

The authors thank the HUSLAB Endocrinology and Metabolism Laboratory staff for sample donation and for their expert technical assistance. We thank Mira Utriainen for expert technical assistance.

References

[1] M. Singer, C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, et al., The third international consensus definitions for Sepsis and septic shock (Sepsis-3), JAMA. 315 (2016) 801–810.
[2] D.C. Angus, T. van der Poll, Severe sepsis and septic shock, N. Engl. J. Med. 369 (2013) 2063.
[3] T. Kuga, M. Mohri, K. Egashira, Y. Hirakawa, T. Tagawa, H. Shimokawa, et al., Bradykinin-induced vasodilation of human coronary arteries in vivo: role of nitric oxide and angiotensin-converting enzyme, J. Am. Coll. Cardiol. 30 (1997) 108–112.
[4] D. Regoli, D. Jukic, F. Gobeil, N.E. Rhaleb, Receptors for bradykinin and related kinins: a critical analysis, Can. J. Physiol. Pharmacol. 71 (1993) 556–567.
[5] J. Antonen, I. Leppanen, J. Tenhunen, P. Arvola, S. Makela, A. Vaheri, et al., A severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant, Scand. J. Infect. Dis. 45 (2013) 494–496.
[6] O. Laine, I. Leppanen, S. Koskela, J. Antonen, S. Makela, M. Sinisalo, et al., Severe Puumala virus infection in a patient with a lymphoproliferative disease treated with icatibant, Infect. Dis. (Lond). 47 (2015) 107–111.
[7] A. Vaheri, T. Strandin, A.J. Jaaskelainen, O. Vapalahti, H. Jarva, M.L. Lokki, et al., Pathophysiology of a severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant, Antivir. Res. 111 (2014) 23–25.
[8] Y. Kayashima, O. Smithies, M. Kakoki, The kallikrein-kinin system and oxidative stress, Curr. Opin. Nephrol. Hypertens. 21 (2012) 92–96.
[9] M. Majima, K. Nishiyama, Y. Iguchi, K. Yao, M. Ogino, T. Ohno, et al., Determination of bradykinin-(1-5) in inflammatory exudate by a new ELISA as a reliable indicator of bradykinin generation, Inflamm. Res. 45 (1996) 416–423.
[10] J. Nussberger, M. Cugno, C. Amstutz, M. Cicardi, A. Pellacani, A. Agostoni, Plasma bradykinin in angio-oedema, Lancet. 351 (1998) 1693–1697.
[11] B.H. van Leeuwen, J.A. Millar, M.T. Hammat, C.I. Johnston, Radioimmunoassay of blood bradykinin: purification of blood extracts to prevent cross-reaction with endogenous kininogen, Clin. Chim. Acta. 127 (1983) 343–351.
[12] K. Shimamoto, O. Iimura, Measurement of circulating kinins, their changes by inhibition of kininase II and their possible blood pressure lowering effect, Agents Actions Suppl. 22 (1987) 297–307.
[13] E. Baralla, M. Nieddu, G. Boatto, M.V. Varoni, D. Palomba, M.P. Demontis, et al., Quantitative assay for bradykinin in rat plasma by liquid chromatography coupled to tandem mass spectrometry, J. Pharm. Biomed.Anal. 54 (2011) 557–561.
[14] I. van den Broek, R.W. Sparidans, J.H. Schellens, J.H. Beijnen, Quantitative assay for six potential breast cancer biomarker peptides in human serum by liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. B. Analyt Technol. Biomed. Life. Sci. 878 (2010) 590–602.
[15] D.J. Campbell, Towards understanding the kallikrein-kinin system: insights from measurement of kinin peptides, Braz. J. Med. Biol. Res. 33 (2000) 665–677.
[16] M. Cugno, J. Nussberger, M. Cicardi, A. Agostoni, Bradykinin and the pathophysiology of angioedema, Int. Immunopharmacol. 3 (2003) 311–317.
[17] K.C. KF Seip, H.T. Bjerknes, E.W. Johansen, L. Nielsen, L. Landro, Reubsaet. Bradykinin analysis revived–a validated method for determination of its stable metabolite in whole blood by LC-MS/MS, J. Chromatogr. B. Analyt Technol. Biomed. Life. Sci. 947-948 (2014) 139–144.
[18] U.L. Hulthen, T. Borge, Determination of bradykinin in blood by a sensitive radioimmunoassay, Scand. J. Clin. Lab. Invest. 36 (1976) 833–839.
[19] O. Heudi, C. Ramirez-Molina, P. Marshall, A. Amour, S. Peace, S. McKeown, et al., Investigation of bradykinin metabolism in human and rat plasma in the presence of the dual ACE/NEP inhibitors GW660511X and omapatrilat, J. Pept. Sci. 8 (2002) 591–600.
[20] A. Barratt-Due, H.T. Johansen, A. Sokolov, E.B. Thorgersen, B.C. Hellerud, J.L. Reubsaet, et al., The role of bradykinin and the effect of the bradykinin receptor antagonist icatibant in porcine sepsis, Shock. 36 (2011) 517–523.
[21] M. Cyr, Y. Lepage, C. Blais Jr., N. Gervais, M. Cugno, J.L. Rouleau, et al., Bradykinin and des-Arg(9)-bradykinin metabolic pathways and kinetics of activation of human plasma, Am. J. Physiol. Heart Circ. Physiol. 281 (2001) H275–H283.
[22] A.M. Fein, G.R. Bernard, G.J. Criner, E.C. Fletcher, J.T. Good, W.A. Knaus, et al., Treatment of severe systemic inflammatory response syndrome and sepsis with a novel bradykinin antagonist, deltibant (CP-0127). Results of a randomized, double-blind, placebo-controlled trial. CP-0127 SIRS and Sepsis Study Group, JAMA. 277 (1997) 482–487.