Development Of A Novel Kinin Biomarker Assay For Characterization Of Bradykinin-Mediated Disorders

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Introduction

- Bradykinin (BK) is involved in various physiological and pathological processes, including angioedema (AE).[1] AE is a predominant manifestation in multiple medical conditions and is generally mediated by BK and/or histamine.[2]
- Differentiating BK-mediated from histamine-mediated AE and assessing the role of bradykinin in the pathogenesis of other conditions by measuring kinin peptides remains a challenge due to their proteolytic instability and limitations of current analytical assays.[3]
- In addition, unspecific activation of the plasma kallikrein-kinin system (KKS), resulting in cleavage of high-molecular-weight kininogen (HMWK) and production of kinins, could have a significant impact on the results.[3]
- Establishment of a method to accurately measure BK and related peptides could aid in identifying, studying, and managing BK-mediated disorders.

Materials and Methods

- To inhibit ex vivo activation of KKS proteases and proteolytic degradation of BK, a protease inhibitor (PI) cocktail was manufactured in a liquid form (Liquid PI).
- Blood samples were collected from healthy volunteers (HV) by Fidelis Research AD (Sofia, Bulgaria) in accordance with the Declaration of Helsinki and approved by The National Bioethics Committee of Medicines and Medical Devices (protocol no. FRT-19101). All participants provided their written informed consent before enrolment.
- Plasma was collected into tubes containing either liquid PI or ethylenediaminetetraacetic acid (EDTA) as a control.
- An ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC)-MS/MS protocol was optimized to separately measure BK and related peptides BK1-9, BK1-8, BK1-7, BK1-5, and kallidin (KD).

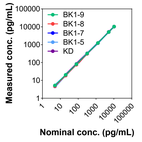
Results

- Calibration curves were prepared by spiking different kinin peptides in surrogate blank matrix.
- For all tested kinins, the back-calculated concentrations of the calibrator standards were within ±15% of the nominal value and met qualification criteria (**Figure 1**).

Figure 1. Calibration curves of analyzed Kinins

Kinin peptide	LLoQ	ULoQ
BK1-9	5 pg/mL	10240 pg/mL
BK1-8	5 pg/mL	10240 pg/mL
BK1-7	5 pg/mL	10240 pg/mL
BK1-5	5 pg/mL	10240 pg/mL
KD	20 pg/mL	10240 pg/mL

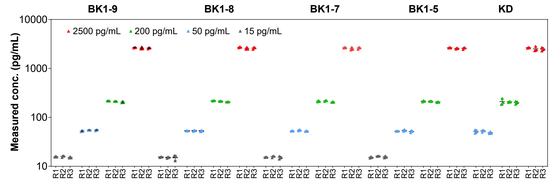
LLoQ: lower limit of quantification; ULoQ: upper limit of quantification



Results

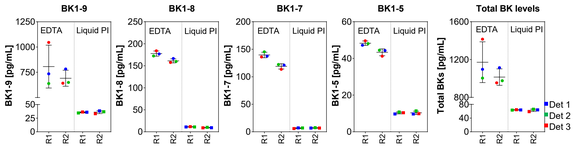
- Kinin UPLC-MS/MS assay in blank matrix met qualification criteria for intra- and inter-run accuracy and precision.
- Accuracy: ±15 % deviation from nominal value.
- Precision: Coefficient of variation (CV) <15% for (**Figure 2**).

Figure 2. Analysis of quality control (QC) samples for analyzed kinins for BK1-9, BK1-8, BK1-7, BK1-5 and KD in blank matrix



- KD levels were below the limit of detection in all types of human plasma analyzed (not shown).
- Liquid PI efficiently inhibited KKS activation and stabilized levels of all BK peptides analyzed and total BK levels compared with EDTA alone, resulting in low inter-run and intra-run variability (CV <15%) (**Figure 3**).

Figure 3. Inter- and intra-run variability in EDTA and Liquid PI plasma from HVs



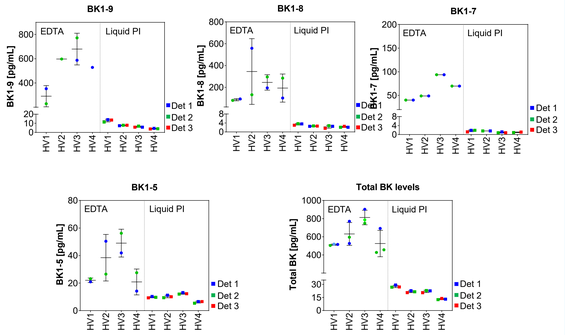
Kinin peptide	EDTA plasma Mean ± SD (pg/mL)	EDTA plasma CV%	Liquid PI plasma Mean ± SD (pg/mL)	Liquid PI plasma CV%
BK1-9	749 ± 81	11	36 ± 0	1
BK1-8	169 ± 12	7	11 ± 1	13
BK1-7	129 ± 15	11	7 ± 0	4
BK1-5	46 ± 3	8	10 ± 0	1
Total BK	1094 ± 111	10	63 ± 1	1

R: Run; Det: Detector; SD: Standard deviation; CV: Coefficient of variation

Results

- EDTA plasma presented higher levels of all BK peptides and high intra-individual variability in plasma from HVs.
- Liquid PI plasma presented low intra-individual variability, CV <15 % for all BK peptides in plasma samples from all HVs (**Figure 4**).
- Liquid PI efficiently inhibited ex vivo KKS activation and kinin degradation.

Figure 4. Intra-individual variability in EDTA and Liquid PI plasma from HVs



HV	EDTA plasma BK1-9 Mean ± SD (pg/mL)	BK1-9 CV%	BK1-8 Mean ± SD (pg/mL)	BK1-8 CV%	BK1-7 Mean ± SD (pg/mL)	BK1-7 CV%	BK1-5 Mean ± SD (pg/mL)	BK1-5 CV%	Total BKs Mean ± SD (pg/mL)	Total BKs CV%
HV1	291 ± 88	30	87 ± 10	12	44 ± 5	12	22 ± 2	7	444 ± 101	23
HV2	2324 ± 2440	105	345 ± 301	87	106 ± 80	76	38 ± 17	44	2813 ± 2839	101
HV3	680 ± 131	19	245 ± 71	29	86 ± 11	13	49 ± 10	21	1060 ± 2224	21
HV4	1231 ± 993	81	193 ± 129	67	58 ± 16	27	21 ± 9	45	1503 ± 1147	76

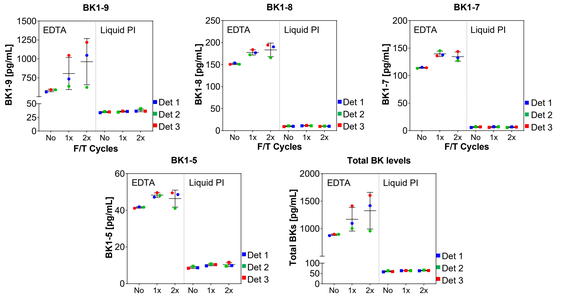
HV	Liquid PI plasma BK1-9 Mean ± SD (pg/mL)	BK1-9 CV%	BK1-8 Mean ± SD (pg/mL)	BK1-8 CV%	BK1-7 Mean ± SD (pg/mL)	BK1-7 CV%	BK1-5 Mean ± SD (pg/mL)	BK1-5 CV%	Total BKs Mean ± SD (pg/mL)	Total BKs CV%
HV1	13 ± 1	11	3* ± 0	na	2* ± 0	na	10 ± 0	5	28 ± 1	5
HV2	8 ± 0	4	3* ± 0	na	2* ± 0	na	10 ± 1	9	22 ± 1	5
HV3	6 ± 1	11	2* ± 1	na	1* ± 0	na	12 ± 1	5	22 ± 1	6
HV4	4 ± 0	9	2* ± 0	na	1* ± 0	na	6 ± 1	11	13 ± 1	5

R: Run; Det: Detector; SD: Standard deviation; CV: Coefficient of variation

Results

- Liquid PI efficiently inhibited ex vivo KKS activation and stabilized BK peptides following 2 cycles of freeze and thaw (F/T) as compared with EDTA (**Figure 5**).
- FT induced KKS activation in EDTA plasma resulting in high levels and variability in BK levels (**Figure 5**).

Figure 5. Effects of Freeze and thaw on BK peptide levels and stability



Kinin peptide	EDTA plasma Mean (pg/mL) No FT	EDTA plasma Mean (pg/mL) 1xFT	EDTA plasma Mean (pg/mL) 2xFT	EDTA plasma Deviation % 1xFT	EDTA plasma Deviation % 2xFT	Liquid PI plasma Mean (pg/mL) No FT	Liquid PI plasma Mean (pg/mL) 1xFT	Liquid PI plasma Mean (pg/mL) 2xFT	Liquid PI plasma Deviation % 1xFT	Liquid PI plasma Deviation % 2xFT
BK1-9	582.4	806.4	962.9	38.5	65.3	34.7	35.6	37.9	2.5	8.9
BK1-8	151.7	177.7	183.4	17.1	20.9	10.0	11.3	10.0	13.5	0.5
BK1-7	114.2	139.6	134.5	22.2	17.7	6.5	6.7	6.4	1.6	-3.0
BK1-5	41.6	48.3	46.4	16.1	11.6	8.9	10.3	10.4	15.7	16.8
Total BK	889.9	1172.0	1327.2	31.7	49.1	60.2	63.9	64.6	6.2	7.4

Det: Detector; FT: freeze/thaw

Conclusions

- **The Liquid PI cocktail was shown to be effective in inhibiting non-specific activation of KKS and generation of BK1-9, as compared to EDTA without PI.**
- **The established qualified kinin UPLC-MS/MS biomarker assay can be used to reliably measure kinins in human plasma.**
- **The developed kinin biomarker assay could become a key tool for identifying, studying, and managing BK-mediated diseases, including BK-mediated AE.**

References

1. Kaplan AP, et al. Adv Immunol. 2014;121:41-89. **2.** Maurer M, et al. Clin Rev Allergy Immunol. 2021;61:40-9. **3.** Kaplan AP, et al. Front Med (Lausanne). 2017;4:206.

COI:E.P.: employee of Pharvaris, holds stock in Pharvaris; **O.D.:** CEO of Attoquant Diagnostics GmbH; **D.S.:** principle at Sexton Bio Consulting, LLC, consultant to Pharvaris; **G.Z.:** employee of Globalization Partners GmbH, consultant to Pharvaris, holds RSU; **A.L.:** employee of GrayMatters Consulting and consultant to Pharvaris, holds stocks/stock options in Pharvaris; advisor to Kosa Pharma. Funding statement: Funding towards this study was provided by Pharvaris.