LC-MS/MS Based Biochemical Evaluation of the Renin-Angiotensin-System

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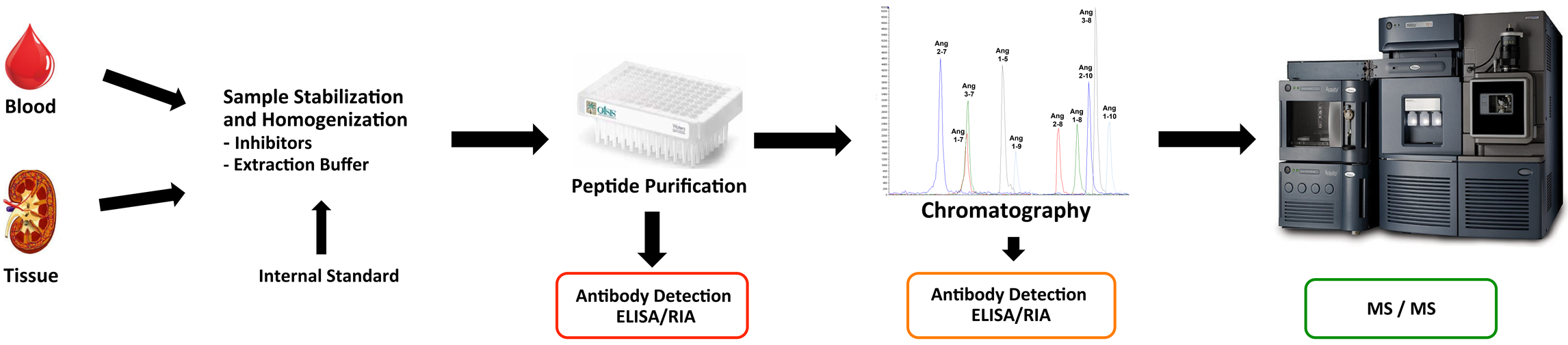
Overview

The accurate quantification of angiotensin peptides is of major importance for research in the field, which represents a huge technical challenge due to extremely low concentrations and a complex sample matrix. The common approaches for angiotensin quantification either involve antibody based (ELISA, RIA) or direct detection of peptides (LC-MS/MS). Both technologies share the need for efficient sample stabilization and appropriate sample handling prior sample preparation and peptide extraction, which is crucial before final steps of analysis reading assay specific signals (colour, radioactivity, mass-to-charge ratio). The sample analysis process for angiotensin quantification in tissue and blood samples is illustrated and compared in terms of technical and economic features and principle for direct MS/MS based peptide detection is explained. Different LC-MS/MS based assays for biochemical evaluation of the RAS were used to characterize a cohort of 12 human healthy volunteers, analyzing plasma and serum samples for angiotensin peptide levels, renin activity, active angiotensinogen, aldosterone, ACE, ACE2 and PCP activity.

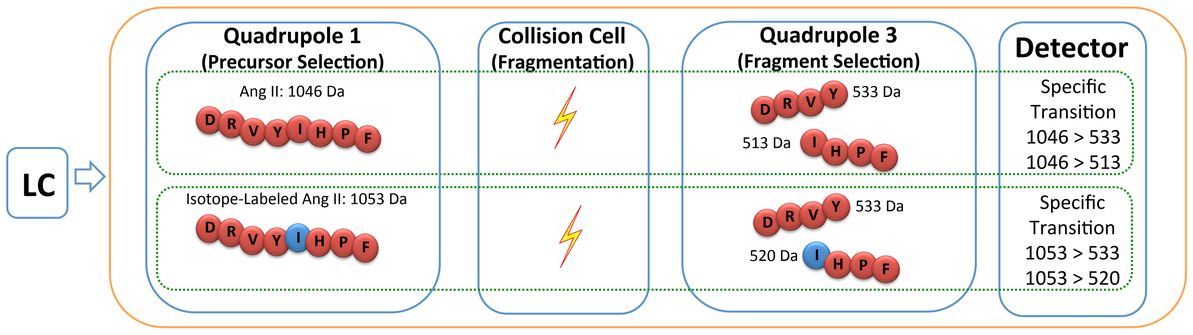
Angiotensin Quantification: Workflow



Assay Comparison

	Antibody-based Asssay in Extract	Antibody-based Assay with Chromatography	LC-MS/MS
Sensitivity	High	High	High
Background	High	Medium	Low
Internal Standards	Limited	Limited	Chemically Identical
Equipment Costs	Low	Medium	High
Work Effort	Low	Medium	HIgh
Analytes per Analysis	1	1	up to 50

Tandem Mass Spectrometry



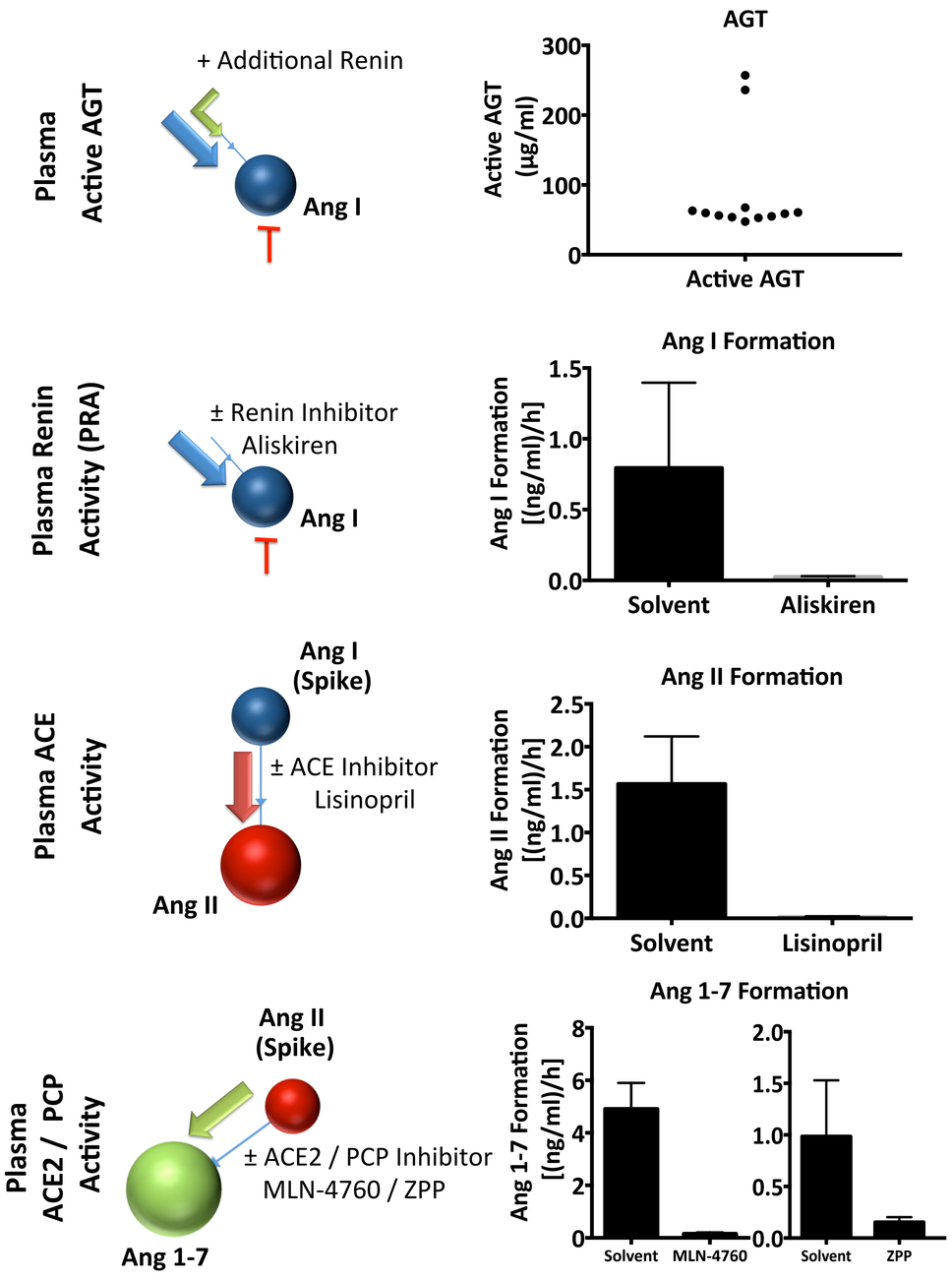
- Major advantage of LC-MS/MS is the simultaneous and specific detection of chemically identical isotope-labeled internal standards within each sample controlling for recovery and matrix effects

Applications for LC-MS/MS based RAS-Analysis

	Plasma Equilibrium		Serum Equilibrium		Circulating Plasma Levels		PRA	ACE	ACE2	PCP	AGT	Aldosterone
	Ang II (pg/ml)	Ang I (pg/ml)	Ang II (pg/ml)	Ang I (pg/ml)	Ang II (pg/ml)	Ang I (pg/ml)	Ang I [(ng/ml)/h]	Ang II [(ng/ml)/h]	Ang 1-7 [(ng/ml)/h]	Ang 1-7 [(ng/ml)/h]	(µg/ml)	(pM)
Donor 1	137,5	64,9	146,4	63,3	15,6	12,5	0,85	1,50	5,08	0,91	58,8	532,6
Donor 2	56,0	41,1	45,8	39,3	3,4	4,1	0,32	1,13	9,67	0,69	60,5	36,0
Donor 3	198,3	119,0	184,9	148,0	14,1	39,3	1,42	1,66	5,54	0,74	55,0	232,9
Donor 4	40,2	50,9	44,0	40,7	2,7	3,4	0,31	0,72	6,45	2,28	59,7	184,5
Donor 5	91,5	62,5	78,3	51,8	2,4	10,0	0,85	1,62	5,46	0,64	56,1	171,9
Donor 6	71,8	69,1	71,9	42,7	6,7	8,5	0,55	1,79	4,66	0,47	47,5	151,4
Donor 7	158,6	131,3	115,2	108,5	8,7	19,3	1,28	1,25	5,39	0,56	63,0	659,4
Donor 8	28,7	6,3	26,7	8,6	0,6	3,1	0,29	2,99	3,76	0,64	53,8	88,8
Donor 9	48,8	42,7	61,2	40,6	4,5	5,2	0,45	1,22	4,41	0,81	52,8	21,7
Donor 10	134,3	74,6	114,1	38,1	4,4	7,9	0,71	1,88	4,09	0,48	67,4	387,6
Donor 11	175,1	123,1	182,0	109,1	16,6	31,4	2,26	1,66	4,20	1,23	256,9	388,1
Donor 12	32,0	22,5	44,0	25,7	3,5	7,1	0,24	1,39	3,64	0,32	236,0	389,6
Mean	**97,8**	**67,3**	**92,9**	**59,7**	**6,9**	**12,7**	**0,79**	**1,57**	**5,20**	**0,81**	**89,0**	**270,4**
SD	60,3	39,6	55,0	40,9	5,5	11,6	0,60	0,55	1,64	0,52	73,9	201,0
LLOQ	*1,0*	*1,5*	*1,0*	*1,5*	*1,0*	*1,5*	*0,05*	*0,03*	*0,15*	*0,15*	*0,75*	*20*
Min. Sample Volume	200 µl						100 µl	5 µl	20 µl	20 µl	5 µl	200 µl

Technical Aspects

- Blood sampling in upright position (no resting)
- Protease Inhibitor Stabilization (Circulating Ang Levels)
  - EDTA, Pepstatin A, p-Hydroxymercuribenzoic acid, Phenanthrolin, AEBSF and specific inhibitors for Renin, APA and APN
- Plasma and Serum Equilibrium Ang-Levels (60min/37°C/pH=7,4)
- Active AGT: Ang I formation in excess of recombinant renin (pH=7,4/60min/37°C)
- PRA: Ang I Formation (pH=7,4/60min/37°C)
- Enzyme Activities (ACE2, ACE, PCP): Natural substrate conversion (60 min/37°C/pH=7,4)
  - +/- specific inhibitors
- Aldosterone: Internal standard controlled LC-MS/MS based assay



Summary

In a cross-sectional approach, we performed a detailed biochemical characterization of the RAS in 12 healthy human volunteers, employing a internal standardized and well-controlled process covering sampling, sample preparation and LC-MS/MS based quantification. Active angiotensinogen concentrations narrowly ranged between 47 and 67µg/ml, except for two individuals with nearly 5-fold increased levels, which might indicate possible AGT-polymorphisms. Mean aldosterone levels were 270 pM, which might be caused by sampling in the upright position and a lack of a resting period prior blood sampling. Minor differences between angiotensin equilibrium levels in serum and plasma were detected, while a solid correlation between circulating and equilibrium angiotensin levels was found. ACE, ACE2 and PCP activities were situated in a narrow range, while PRA and angiotensin levels were subject to significant donor specific variations.

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