Simultaneous Determination of Angiotensin and Bradykinin Metabolites in Human Plasma/Serum by LC/MS/MS Analysis

Description:

Purpose

Angiotensin is a peptide hormone that causes vasoconstriction and increase in blood pressure; it has been shown to be the culprit in hypertension, CVD, diabetes, etc.. As a precursor and renin substrate, angiotensin is cleaved at the N-terminus by renin resulting in angiotensin I, which will later be metabolized by ACE (Angiotensin-Converting Enzyme) peptidases, to become angiotensin II (Ang II), and metabolized further by aminopeptidase A, into Ang III and then into Ang IV [10]. In addition, Ang II is metabolized alternatively by a second form of ACE peptidases (ACE2-regulatory enzyme) into novel peptides angiotensin 1-9, and then by ACE into angiotensin 1-7.

Clinical findings in COVID-19 patients mainly point to increased activity of angiotensin II and the corresponding lack of angiotensin 1-7 activity suggesting a role of ACE2 blockade in its pathogenesis. There was an urgent need for assessing the levels of angiotensin 1-7 in COVID-19 to understand its role in the pathology.

Bradykinin (Kallidin I or 9) is a nonapeptide and a kinin generated from kininogen by the action of protease during tissue injury; conversely with angiotensin II, bradykinin promotes blood vessel dilation and a lowering of blood pressure considered to play a part in inflammatory processes and a relevant role in COVID-19. Bradykinin is degraded in human plasma by a carboxypeptidase to yield a metabolite of des Arg 9 - bradykinin (**DABK**).

Kallidin a decapeptide vasodilator consisting of bradykinin with a lysyl group attached to amino terminus is a kinin produced by action of tissue and glandular kallikreins on low-molecular weight kininogen (LMWK) and having physiologic effects to those of bradykinin.
Kallidin can be degraded either by an aminopeptidase in the blood to yield bradykinin or by carboxypeptidase to be des-arginine kallidin (D**AKD**). Kinin peptide metabolism is an important determinant of kinin levels in blood and tissue. The assessment on metabolites of angiotensin (Ang II and Ang 1-7) and bradykinin (DABK and DADK) is useful for understanding inflammatory processes and pathophysiology of COVID-19 related to the Kallikrein–Kinin (KKS), the Coagulation/Fibrinolysis, and the Renin–Angiotensin (RAS) Systems

Method

Angiotensin II and Angiotensin 1-7, DABK and DAKD in human plasma/serum are extracted by Protein Precipitation (PP) and Solid Phase Extraction (SPE), separated and eluted by High Performance of Liquid Chromatography (HPLC), and determined by Mass Spectrometry (MS) in Electrospray Ionization (ESI) source at positive ionization mode with multiple reaction monitoring (MRM) of transitions. Stable isotope labeled Angiotensin II, Angiotensin 1 -7, DABK, and DADK are utilized as internal standards for the calibration of Angiotensin II, 1-7, DABK and DADK metabolite assays.

Collection and Performance Characteristics

Sample Type	Preferred: SST Alternate: Plasma
Minimum Volume	0.5 mL
Special Processing Considerations	Store at -80°C until analysis is performed Avoid repeated freeze-thaw cycles
Lowest Reportable Value:	2 pg/mL (Ang II, Ang 1-7, and DAKD) 20 pg/mL (DABK)
Dynamic range	2 - 500 pg/mL (Ang II, Ang 1-7, and DAKD) 20 - 5000 pg/mL (DABK)
Precision	Intra-assay variation is 4.7 - 13.4% Inter-assay variation is 4.1 - 14.7%
Reference Range	Unknown
Note	A specific individual test of Ang II, Ang 1-7, DABK, and DADK is also acceptable