Studies on Histamine Synthesis in Mouse Maternal Blood and Cultured Cells (Abstract)

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Preface

Living organisms contain various bioactive macromolecules such as proteins and nucleic acids, as well as bioactive small molecules like amino acids, vitamins, and hormones. These molecules function cooperatively to maintain homeostasis. The rapid development of various metabolomic techniques has underscored the importance of investigating small, endogenous, bioactive molecules for understanding the homeostasis and pathogenesis of higher-level physiological functions.

In particular, the amounts of bioactive small molecules in maternal body are altered significantly during pregnancy. Recent dramatic changes in lifestyle have led to an increase in the prevalence of various complications of pregnancy. During pregnancy, some of bioactive amines, such as monoamines, diamine and peptides, which commonly contain primary or secondary amino groups, have been known to be involved in the maintenance of blood pressure homeostasis, and variations in their concentrations have been observed in various cardiovascular diseases. As an example of these bioactive amines, angiotensin II (AngII), a potent vasoconstrictor derived from renin-angiotensin system, is a major regulative amine of blood pressure, not only under normal conditions but also under pregnant state.

Pregnancy-induced hypertension (PIH) is a common complication that occurs in approximately 10% of human pregnancies. Pregnant women with PIH may display transient blood pressure elevation, proteinuria, cardiac hypertrophy, edema, organ dysfunction, intrauterine growth retardation (IUGR), and perinatal death in late pregnancy. This syndrome places both mother and fetus at high risk; however, its pathogenic mechanism is still unclear despite numerous clinical studies. Further investigation has been hindered by ethical issues involved in research on PIH patients and technical limits derived from the lack of an appropriate animal model for this disease.

It has recently been theorized that AngII was one of causes in the pathogenesis of PIH. Our laboratory previously developed a transgenic mouse model in which pregnancy-associated hypertension (PAH) developed due to the overproduction of AngII in the maternal circulation during late pregnancy. PAH mice showed a transient elevation of blood pressure, proteinuria, cardiac hypertrophy, IUGR, and perinatal death in late gestation. These gestational disorders are caused by excessive AngII – type I angiotensin II receptor (AT_1R) signaling. However, the mechanisms involved in the downstream of the AngII–AT1R system are unknown.

I hypothesized that the pathogenesis of PIH would be affected by disruption of the quantitative balance of bioactive amines during pregnancy. I used PAH mice as a tool for studies on human PIH, and tried to explore

and identify the bioactive amines involved in the pathogenesis of PAH.

Results

HPLC analyses of plasma through detections of amino group containing substances from both WT and PAH mice at day 19 of gestation revealed that the amount of a specific peak at 15.5 min from PAH mice was relatively higher than that from WT mice. Following this screening, I performed mass analysis with MALDI-QIT-TOF/MS and structural analysis with ^{1}H NMR. The isolated molecule had a molecular mass of 111.25 and contained an imidazole ring in its structure. I searched for a candidate substance using the Spectral Database for Organic Compounds (SDBS), and revealed that histamine was the primary candidate amine. The retention time of commercial histamine standard was identical to that of the identified amine on the HPLC chromatogram. Furthermore, MS and MS/MS spectra of histamine corresponded to that of the target amine. These results suggest that the characterized substance as an elevated amine in PAH mice was histamine. After I established the measuring method for trace amounts of histamine in cultured cells by using ultra-high performance liquid chromatography (UPLC), I applied it for absolute quantitative analysis of histamine in mice plasma, and revealed that plasma histamine levels of PAH mice were 1.7-fold significantly higher than that of WT mice.

Discussion

Pregnancy-induced hypertension (PIH) is a high-risk disease for both mother and baby; however, the underlying pathogenic mechanisms of PIH remain to be solved despite many intensive clinical investigations. Identifying these mechanisms will contribute greatly to the development of effective therapies.

I focused on small molecules essential for progression of gestation, and explored the bioactive amines involved in the pathogenesis of PAH using HPLC, MALDI-QIT-TOF/MS, and ^{1}H NMR. The results of these analyses showed that plasma histamine levels in PAH mice were higher than those in WT mice at day 19 of gestation.

Histamine is well known to be involved in a variety of physiological responses, such as allergy, inflammation, gastric acid secretion, and neurotransmission. Histamine is synthesized by histidine decarboxylase (HDC) with L-histidine as a specific substrate. On the other hand, it is metabolized in two ways: one is via oxidative deamination by diamine oxidase (DAO), and the other is via imidazole ring-methylation by histamine-*N*-methyltransferase (HNMT), an enzyme that converts histamine to 3-methyl histamine only in the cytoplasm. It has been reported that histamine levels are elevated during the third trimester in pregnant

women with PIH, and the clinical manifestations of PIH resemble those of hyperhistaminemia. It has also been reported that histamine may inhibit apoptotic activity in trophoblast cell cultures via the H1 receptor. Although the relationship between histamine and PIH was investigated in the 1950s, the functions of histamine in PIH have not been fully examined. Because experiments in PIH patients conflict with ethical guidelines, we could not conduct further investigations.

In the present study, I was not able to clarify whether or not histamine plays a causal role in PAH. By using PAH mice, we can perform future studies on human PIH without ethical limitations. Future analyses of PAH mice will help to elucidate the molecular mechanisms underlying human PIH and lead to the development of new therapies.