Phenylacetylglutamine causes a pathologic inflammation state and enhances atherosclerosis through the b2-adrenergic receptor cAMP PKA NF-kappaB pathway in diabetes

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Background: Type 2 diabetes (T2DM) affects billions of patients and has a pathophysiologically important inflammatory component. Macrophage plasticity in diabetes is impaired, and remains in a persistent proinflammatory state; yet currently, the cause of the inflammatory state is unknown. Phenylacetylglutamine (PAGln) is an amino acid metabolism end product that rises in T2DM patients and is associated with incident major adverse cardiovascular events. However, the role of PAGln in the inflammatory component of T2DM remains unknown.

Purpose: Here, we aim to identify blood-based systemic factors for pathologic inflammation status and risk of atherosclerosis as they are highly reproducible at low cost and search for effective and safe agents to attenuate diabetic inflammatory complications.

Methods: In vivo, phenylacetylglutamine was dissolved in normal saline and injected in mice daily (intraperitoneal). The levels of PAGln were analyzed by liquid chromatography-mass spectrometry. The effect of PAGln on atherosclerosis progression was analyzed by Ldlr/−/− mice, followed by a Western diet feeding for 12 weeks. In vitro, primary mice bone marrow-derived macrophages (BMDMs) were treated with phenylacetylglutamine (100 uM) for the indicated time. Elisa, PCR, and western blot were used to characterize the impact of PAGln on BMDMs and mice arteries. Flow cytometry was taken to quantify and type immune cells in mice bone marrow, blood, spleens, and arteries. The activity of the NF-κB pathway was assessed by KEGG analysis, western blot, luciferase, and activity assay.

Results: In vivo, increased inflammatory macrophages were detected in mice blood, spleens, bone marrow, and arteries after PAGln injection. In vitro, PAGln leads to macrophage M1 polarization. Taken together, PAGln disturbed the transition of macrophage phenotype from inflammatory to reparative, facilitating pathologic inflammation. The RNA of BMDMs after PAGln treatment was collected for RNA-seq. KEGG analysis and western blot prove PAGln exerted its function through the activation of NF-κB. PAGln could promote NF-κB signals by increasing the phosphorylation level of p65. Besides, the negative effects of PAGln could be rescued by β-blockers.

Conclusion: Our study unravels a novel mechanism by which PAGln accelerates atherosclerosis progression and causes pathologic inflammation by activating the b2-adrenergic receptor/cAMP/PKA/NF-κB pathway. The negative effect of PAGln could be rescued by carvedilol (β blocker). Thus, our recognition of chronic inflammation in diabetes needs to be revised, and understanding these mechanisms can inform alternative treatment strategies and refine atherosclerosis repair therapies for diabetic patients.