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Molecular mechanisms underlying anti-inflammatory and antioxidant properties of gallic acid and *Terminalia bellirica* extract

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Lifestyle-related diseases such as obesity, diabetes and dyslipidemia due to excessive energy intake and lack of exercise, and atherosclerosis associated with these diseases are the principal cause of death around the world. Macrophage-mediated inflammation and oxidative stress play an important role in the initiation and progression of these disorders. Upon stimulation, macrophages release inflammatory mediators including pro-inflammatory cytokines, nitric oxide (NO) and reactive oxygen species (ROS), which contribute to tissue damage and the development of inflammation-related diseases. Furthermore, in recent years, obesity-induced chronic inflammation in adipose tissue is known to cause various lifestyle-related diseases as a contributing factor to metabolic disorders such as insulin resistance and dyslipidemia. Therefore, the inhibition of excess inflammation and oxidative stress in macrophages and chronic inflammation in adipose tissue is important for providing a therapeutic strategy against inflammation-related diseases.

Dietary antioxidants including polyphenols have been reported to exert an antioxidant and anti-inflammatory activities. *Terminalia bellirica* extract (TBE) is obtained from the fruit of *Terminalia bellirica* tree, which is distributed throughout Southeast Asia and used as a folk medicine for diabetes, rheumatism, and hypertension in traditional Indian Ayurvedic medicine. We previously reported that TBE inhibited inflammation and ROS production in THP-1 macrophages and that approximately 50% of the polyphenolic compounds contained in TBE is gallic acid (GA). GA is a natural polyphenol that is found widely distributed in fruits and plants. GA has higher bioavailability than other polyphenols, and *in vitro* and *in vivo* studies have shown anti-inflammatory, anti-obesity and hypoglycemic properties of this polyphenol. However, molecular mechanisms underlying anti-inflammatory and antioxidant activities of GA and TBE remain unclear. In the present study, we investigated the effects of GA and TBE on inflammation and oxidative stress, as well as the underlying molecular mechanisms.

First, we examined the inhibitory effects of GA and TBE on inflammation and oxidative stress by utilizing lipopolysaccharide (LPS)-stimulated macrophages and LPS-shock model mice. We found that GA and TBE attenuated LPS-induced inflammatory mediator expression, NO production, ROS production, and activation of mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) in macrophages. Furthermore, GA and TBE increased antioxidant enzyme expression along with upstream mediators nuclear factor erythroid-2-related

factor 2 (Nrf2), Akt, and AMP-activated protein kinase (AMPK). Importantly, knockdown of Nrf2 by siRNA and specific inhibition of Akt and AMPK significantly reduced antioxidant enzyme expression induced by GA and TBE. We also assessed *in vivo* effects on histopathology and gene expression in tissues collected after intraperitoneal injection of LPS with or without TBE treatment. TBE enhanced antioxidant enzyme expression and improved acute kidney injury in LPS-shock model mice. These results suggest that GA and TBE exert protective effects against inflammation and oxidative stress by suppressing MAPK/NF-κB pathway and by activating Akt/AMPK/Nrf2 pathway.

Next, we examined the inhibitory effect of GA on obesity-induced chronic inflammation by utilizing adipocytes, co-culture of adipocytes and macrophages and diet-induced obese mice. We showed that GA enhanced adipocyte differentiation in 3T3-L1 adipocytes. Consistent with enhancement of adipogenesis, GA decreased gene expression of monocyte chemoattractant protein-1 (MCP-1) and increased that of adiponectin along with upstream mediator, peroxisome proliferator-activated receptor gamma (PPARγ). GA also reduced inflammatory mediator expression induced by co-culture of adipocytes with RAW 264 macrophages. Moreover, diet-induced obese mice treated with GA showed decreased serum cholesterol levels and adipocyte size with improvement in insulin sensitivity without changes in body weight. Decreased expression of inflammatory mediator and macrophage specific marker was also observed in adipose tissue of GA-treated mice. These results suggest that GA suppresses adipocyte hypertrophy and inflammation caused by the interaction between adipocytes and macrophages, thereby improving adipose tissue inflammation and metabolic disorders such as insulin resistance and dyslipidemia.

In conclusion, our present study revealed the molecular mechanisms of GA and TBE against inflammation and oxidative stress. The *in vivo* effects of GA and TBE were partly confirmed, suggesting that GA and TBE might be effective for the treatment of inflammation-related diseases.